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ANALYSIS OF PHENOLS AND ANILINES IN ENVIRONMENTAL AND BIOLOGICAL SAMPLES

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "Analysis of Phenols and Anilines in Environmental and Biological Samples" submitted by Erika E. Hargesheimer in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Pharmaceutical Sciences (Environmental Toxicology).

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DEDICATION

TO MY MOTHER

We are now coming to realize that many of our most pressing problems require a competence in trace organic analysis.

Stephen N. Chesler
Harry S. Hertz
Center for Analytical Chemistry
National Measurement Laboratory
National Bureau of Standards
Washington, D.C.

ABSTRACT

Gas-liquid chromatographic methods have been developed which provide sensitivity and specificity for the analysis of a wide variety of phenolic and anilino compounds of environmental, industrial and medicinal interest. Over forty chloro-, nitro-, alkyl- and polyhydric phenols, hydroxy derivatives of polyaromatic compounds such as 1-naphthol, as well as alkaloid drugs such as morphine, were reacted with acetic or propionic anhydride directly in basified ($NaHCO_3$) aqueous solution. The acyl ester derivatives were then quantitatively extracted into methylene chloride at nanomolar concentration levels. Similarly, a method for the analysis of aminophenols and substituted anilines in aqueous samples was developed involving a two-step derivatization procedure. Acyl derivatives of the amines, also prepared by direct aqueous acylation, were quantitatively extracted into methylene chloride and further reacted with trifluoroacetic anhydride to produce highly electron capture sensitive derivatives. Electron capture sensitive derivatives of morphine were prepared in alkaline (Na_2CO_3) aqueous solution by reaction with pentafluorobenzoyl chloride using acetonitrile as a phase transfer reagent.

The acylated anilino and phenolic compounds, readily separated on packed or capillary gas-liquid chromatographic columns, were identified and characterized using flame ionization detection, electron capture detection, electron impact- and chemical ionization-mass spectrometry as well as selected ion monitoring-mass

spectrometry. The developed procedures were successfully applied to the analysis of trace anilino and phenolic residues in river waters, industrial wastewaters, drinking water, melted snow, as well as human urine and forensic samples.

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LIST OF ABBREVIATIONS

4-AAP	4-aminoantipyrine
ANI	aniline
AP	aminophenol
BA	benzylamine
BSA	N,O-bis(trimethylsilyl)acetamide
BSTFA	N,O-bis(trimethylsilyl)trifluoroacetamide
BrA	bromoaniline
BrP	bromophenol
CA	chloroaniline
CAA	chloroacetic anhydride
CI	chemical ionization
CI-MS	chemical ionization - mass spectrometry
CLE	continuous liquid extraction
3-Cl-4-MeA	3-chloro-4-methylaniline
CRE	cresol
DAI	direct aqueous injection
4,6-DBC	4,6-dibromo-o-cresol
DCAA	dichloroacetic anhydride
DCP	dichlorophenol
DEGS	diethylene glycol succinate
DMP	dimethylphenol
DNP	dinitrophenyl

ECD	electron capture detector (detection)
EI	electron impact
EI-MS	electron impact - mass spectrometry
EPA	Environmental Protection Agency (U.S.A.)
EtP	ethylphenol
FID	flame ionization detector (detection)
FPD	flame photometric detector (detection)
GCB	graphitized carbon blacks
GLC	gas-liquid chromatography (chromatographic)
GPC	gel permeation chromatography
HFBA	heptafluorobutyric anhydride
HFBI	heptafluorobutyrylimidazole
HMDS	hexamethyldisilazane
HPLC	high performance liquid chromatography (chromatographic)
hr	hour (hours)
kg	kilogram (kilograms)
L	litre (litres)
m	metre (metres)
MCP	monochlorophenol
mg	milligram (milligrams)

min	minute (minutes)
mL	millilitre (millilitres)
MS	mass spectrometer (spectrometry)
NAP	naphthol
NCI	negative chemical ionization
NCI-MS	negative chemical ionization - mass spectrometry
ng	nanogram
nm	nanometer (nanometers)
nmole	nanomole (nanomoles)
NP	nitrophenol
PCBs	polychlorinated biphenyls
PCP	pentachlorophenol
PHE	phenol
PFBC	pentafluorobenzoyl chloride
PFPA	pentafluoropropionic anhydride
pg	picogram (picograms)
pmole	picomole (picomoles)
ppb	parts per billion
ppm	parts per million
SIM	selected ion monitoring
SIM-MS	selected ion monitoring - mass spectrometry

TCP	trichlorophenol
TeCP	tetrachlorophenol
TFAA	trifluoroacetic anhydride
TLC	thin-layer chromatography
TMAA	trimethylanilinium acetate
TMCS	trimethylchlorosilane
TMS	trimethylsilyl
TMSA	trimethylselenium acetate
TMSH	trimethylselenium hydroxide
µg	microgram (micrograms)
µmole	micromole (micromoles)
UV	ultraviolet
V	volts

CHAPTER I

INTRODUCTION

New measurement methods for the detection of trace organic residues in complex biological, environmental and forensic samples are constantly being developed. Much attention has been focused on the analysis of phenolic and anilino compounds of both industrial and medicinal importance. Phenols and anilines have been detected in sewage effluents, industrial discharges, natural surface waters, human tissues and biological fluids. The severe restrictions placed on the use of chlorinated pesticides such as DDT, lindane and dieldrin have prompted an increased use of biodegradable pesticides. Phenols and anilines are decomposition products of many of these pesticides. It has been demonstrated that the determination of urinary phenolic and anilino metabolites is a sensitive and specific method for monitoring exposure to the biodegradable pesticides. Many drugs contain phenolic moieties; phenolic groups are also often formed during drug metabolism. The medicinal and forensic importance of morphine and related alkaloid drugs, for example, has stimulated considerable interest in the analysis of these compounds. Despite the urgency, many particularly challenging analytical problems associated with phenolic and anilino compounds remain unresolved. There is a continuous and evergrowing need for the development and improvement of techniques for the sensitive and specific analysis of these compounds.

CHAPTER II

OBJECTIVES

At the present time, many methods are available for the gas-liquid chromatographic (GLC) analysis of phenols and anilines. The quantitative GLC determination of these compounds at low concentrations is hampered by on-column adsorption and decomposition as well as peak tailing. Consequently, most procedures involve a preliminary extraction step followed by a derivatization of the compounds of interest in organic solvent. While techniques for the preparation of derivatives with excellent GLC properties are numerous, these methods must be evaluated based on the efficacy of the analytical procedure as a whole. A method cannot be considered entirely satisfactory if any of the steps in the procedure are inadequate. The recoveries of many phenol and aniline residues from sample matrices by various liquid-liquid extraction, adsorption or distillation techniques are notoriously poor.

The study described in this thesis was initiated to determine whether an analytical method could be found which combines efficiency of extraction with sensitivity of detection and quantitation using GLC. The use of acyl anhydride reagents for the derivatization of phenols and anilines directly in aqueous solution was examined. In order to define the capabilities of the method, acyl derivatives of morphine and alkaloid drugs, aniline and haloanilines, as well as a wide range of alkyl-, halo-, nitro- and aminophenols were prepared. The suitability of acyl derivatives for the quantitation of trace levels of these compounds in aqueous solutions was assessed in terms of extraction efficiency, derivative stability and GLC resolution and response characteristics. Derivatization reactions of compounds

which contain several functional groups, such as the alkaloid drugs and aminophenols, offer invaluable information concerning the scope of a derivatization method and its compatibility with other structures present.

A useful analytical procedure must be capable of quantitatively measuring concentrations of phenols and anilines in samples containing many, more abundant interferences. It was essential, therefore, to determine whether the developed direct acylation techniques could be applied to natural samples containing a variety of organic constituents. A second major objective of this study was the isolation, separation, identification and quantitation of a wide variety of phenolic and anilino compounds in biological, environmental, forensic and industrial samples.

CHAPTER III

LITERATURE SURVEY

A. INTRODUCTION

Anilines and phenols are common industrial and agricultural chemicals whose use, occurence, toxicity, metabolism and degradation have been extensively reviewed (1-18). These compounds have been identified in drinking water, rivers, sewage, industrial waste, aquatic biota, human tissues and biological fluids. Phenol (PHE), cresols (CREs) and many alkylphenols are discharged by coal conversion plants (19-21), coking industries (22-23), petroleum refineries (24-27) and petrochemical plants (25). Dimethylphenols (DMPs), CREs and PHE have also been identified in car exhaust (28, 29). Wastewaters from pulp and paper industries contain detectable levels of many simple dihydric, alkyl- and methoxyphenols (22, 25, 30-34). These constituents may become chlorinated during chlorine bleaching processes. Jolley *et al.* (35) found that chlorophenols were also formed during treatment of secondary sewage by chlorination. Chlorophenols (5) are widely used as fungicides, antimicrobials, wood preservatives and intermediates in the synthesis of many agricultural pesticides. At parts per million (ppm) levels, toxic effects of phenolic compounds have been demonstrated in many species of bacteria, fungi, protozoa, algae and fish (1). Even at parts per billion (ppb) levels, phenolics have been linked to undesirable tastes and odours in drinking water (1, 36).

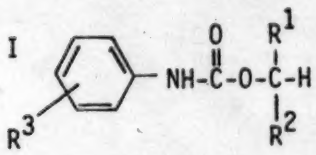
Aminophenols (APs) are common components of hair dyes (13, 22) and both APs and anilines are used in the manufacture of rubber, plastic and textiles (37). Aqueous effluents from coal conversion

processes such as gasification and liquefaction, contain anilines as well as other nitrogenous aromatics in concentrations potentially hazardous to aquatic organisms (14, 15). Aniline (ANI) and substituted anilines are commonly used in the paint, dye and drug industries (38). They are introduced into the environment directly as industrial waste, by the combustion of plastics and urethane products or as the reduced form of nitrobenzene compounds (39).

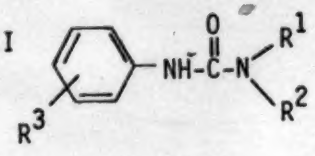
In addition to the industrial sources mentioned above, the degradation of many pesticides contributes to the concentration of 1-naphthol (1-NAP), nitrophenols (NPs) and chlorophenols found in river waters and human urine (40). The degradation of N-phenylcarbamate and N-phenylurea herbicides (Table I) in soils (41-45), environmental water systems and man (18, 40) is the most common source of anilines. Similarly, the phenols shown in Table II are decomposition products of many biodegradable pesticides. Although anilino degradation products have no herbicidal properties (44), they are often more toxic than the parent compounds and persist in the environment strongly bound to soil organic matter (44, 45). In addition, they may undergo further conversion to persistent and carcinogenic azo metabolites (46, 47). Many nitro- and chlorophenols are also highly persistent (48).

The literature dealing with the analysis of morphine and other alkaloid drugs is extensive. Of the hundreds of methods available, only radioactive, spectrofluorometric, GLC and high performance liquid chromatographic (HPLC) methods allow the determination of concentrations in the nanogram (ng) range. Although methods using

Table I. Decomposition of Certain Herbicides to Aniline Degradation Products



N-phenylcarbamate



N-phenylurea

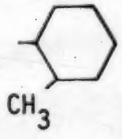
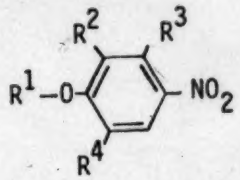
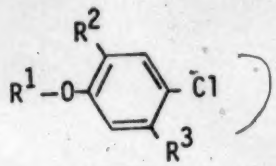
Class	Common Name	R^1	R^2	R^3	Degradation Product
I	Propham (IPC)	$-CH_3$	$-CH_3$	-H	aniline
I	Carbetamide	$-CH_3$	$-\overset{O}{\overset{\|}{C}}NHCH_2CH_3$	-H	aniline
II	Fenuron	$-CH_3$	$-CH_3$	-H	aniline
II	Siduron	-H		-H	aniline
I	Chlorpropham	$-CH_3$	$-CH_3$	3-Cl	3-chloroaniline
I	Barban	$-C\equiv CCH_2Cl$	-H	3-Cl	3-chloroaniline
II	Monolinuron	$-CH_3$	$-OCH_3$	4-Cl	4-chloroaniline
II	Buturon	$-CH_3$	$-CH(CH_3)C\equiv CH$	4-Cl	4-chloroaniline
II	Monuron	$-CH_3$	$-CH_3$	4-Cl	4-chloroaniline
II	Metabromuron	$-CH_3$	$-OCH_3$	4-Br	4-bromoaniline
II	Chlorotoluron	$-CH_3$	$-CH_3$	3-Cl, 4-CH_3	3-chloro-4-methylaniline

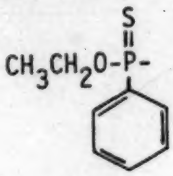
Table II. Decomposition of Certain Pesticides to Phenol Degradation Products



I Nitrophenol pesticides



II Chlorophenol pesticides

Type	Name	R^1	R^2	R^3	R^4	Degradation Product
I	EPN		-H	-H	-H	4-nitrophenol
I	Methyl Parathion	$(CH_3O)_2$-P(=S)-	-H	-H	-H	
I	Ethyl Parathion	$(CH_3CH_2O)_2$-P(=S)-	-H	-H	-H	
I	Fenitrothion	$(CH_3O)_2$-P(=S)-	-H	$-CH_3$	-H	p-nitrocresol
I	Dicapthon	$(CH_3O)_2$-P(=S)-	-Cl	-H	-H	2-chloro-4-nitrophenol
I	2,4-Dinitrocresol Sodium Salt	Na-	$-CH_3$	-H	$-NO_2$	2,4-dinitro-cresol
II	VC-13	$(CH_3CH_2O)_2$-P(=S)-	-Cl	-H	-	2,4-dichloro-phenol
II	2,4-D	$HOCCH_2-$ (=O)	-Cl	-H	-	
II	Fenchlorphos	$(CH_3O)_2$-P(=S)-	-Cl	-Cl	-	2,4,5-Trichloro-phenol
II	2,4,5-T	$HOCCH_2-$ (=O)	-Cl	-Cl	-	
II	Silvex	$HOCCH(CH_3)-$ (=O)	-Cl	-Cl	-	
II	Erbon	$CH_3C(Cl)(Cl)C(=O)O(CH_2)_2-$	-Cl	-Cl	-	

radioimmunoassay (49) or spectrofluorimetry (50, 51) are highly sensitive, they lack specificity. GLC and HPLC have both been used successfully for the unequivocal analysis of morphine and other alkaloid drugs; only the latter two methods will be included in this literature survey.

Sensitive, specific analytical methods are required to measure trace amounts of simple phenols and anilines because of their toxicity (11, 52, 53) and their potential utility as indicators of contamination by biodegradable pesticides. Specific methods are required for the analysis of morphine and related alkaloids in plasma, tissue samples, urine and a wide variety of forensic samples. Rossini, Chairman of the Environmental Measurements Advisory Committee of the U.S.A. Environmental Protection Agency (EPA) is quoted as saying: "There is no substance on earth that has absolute zero of impurity in it, nor can man make it so. If we cannot measure it we know nothing about it, and when we do measure it the resulting figure has associated with it a specific range of uncertainty." (54). Considerable effort has been expended to improve the reliability of methods for the isolation, separation, identification and quantitation of phenols and anilines. At ppb levels, many phenolic and anilino compounds are of little toxicological significance (1, 55), nevertheless, it is important to accurately characterize the substances which do occur in order to evaluate possible effects and dangers. A brief summary of recent developments and procedures currently available is now presented.

B. ANALYTICAL METHODS FOR SIMPLE PHENOLS AND ANILINES

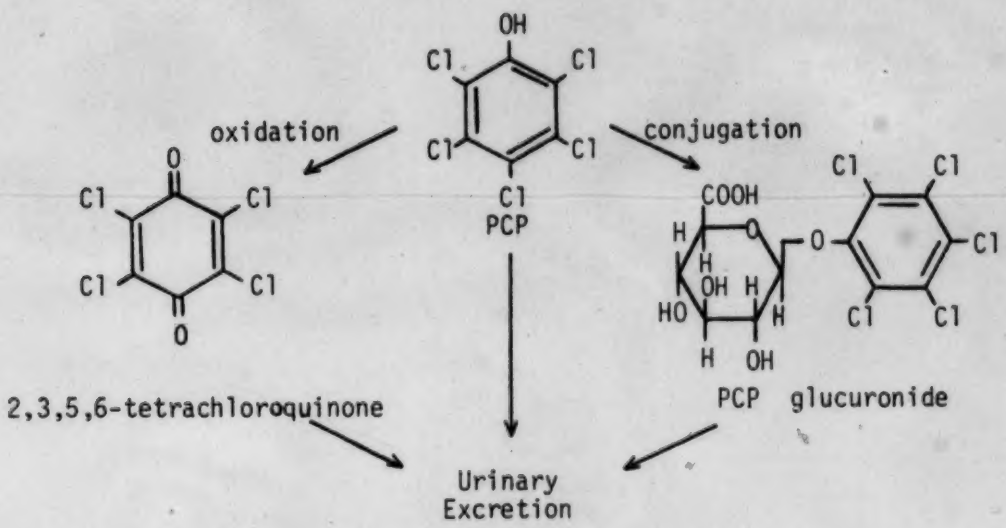
1. Sample Preparation

a) Hydrolysis

Phenols and anilines may be excreted in urine as either salts or conjugates of glucuronic, sulfuric or acetic acid. Conjugates must be converted to the free forms prior to extraction; alkaline, acid or enzymatic hydrolysis procedures are commonly used for most urinary phenols and anilines (40, 56-61). Of the three methods, Duran et al. (57) considered acid hydrolysis superior for the recovery of normal urinary phenols. Prior to 1972, with the exception of the procedure described by Shafik et al. (62), methods used for the analysis of chlorophenols in urine did not include a hydrolysis step. Cranmer and Freal (63) reported that a single hexane extraction of an unhydrolyzed urine sample yielded 90% of the pentachlorophenol (PCP) present and Bevenue et al. (64) claimed that refluxing urine with concentrated acid before extraction did not increase the amount of PCP extracted. In 1974, a hydrolysis step for urine was not included in the EPA method for PCP (65) which endorsed the findings reported by Bevenue et al. In contrast, acid hydrolysis was recommended in EPA procedures for the analysis of 1-NAP (66) and 4-NP (67).

While chlorophenols are primarily excreted in an unconjugated form in the urine, conjugation has been

demonstrated in several species (2, 68-71). Scheme 1 illustrates the major metabolic pathways of PCP in mammals (71).



Scheme 1.

Edgerton and Moseman (72) found that PCP concentrations determined using methods incorporating an acid hydrolysis step were seventeen fold higher than those obtained without hydrolysis. It was reported that an acid hydrolysis time of one hour (hr) was required for maximum recovery of biologically incorporated PCP from urine. Hydrolysis is now considered essential for complete recovery of chlorophenols from urine samples (73-76). In 1979, the EPA revised its method for urine chlorophenol analysis to include an acid hydrolysis step (77).

The rapid method, previously advocated for the analysis of urine, is now recommended only for blood or serum samples.

b) Extraction

Separation of phenolic and anilino residues from a sample matrix can be accomplished using liquid-liquid extraction, continuous liquid extraction, steam distillation or resin adsorption. Many different solvents have been used for extraction, including toluene (78-80), petroleum ether (78), chloroform (25, 79), methylene chloride (25, 76, 82-88), hexane (65, 80, 89-93), acetonitrile (94), diethyl ether (95-97), benzene (72, 74, 93, 95, 98-100), ethyl acetate (101, 102), acetone (103, 104) and a variety of other combinations of solvents (73, 75, 89, 105-109). Choice of solvent is affected by the composition of the sample matrix as well as the type and concentration of the compounds of interest. Ivanov and Magee (96) extracted chlorophenols from water with diethyl ether but extraction efficiencies for PCP and 2,3,5-trichlorophenol (2,3,5-TCP) were much poorer than those achieved using methylene chloride (110). For the removal of phenolics from water samples, Realini (111) investigated a number of solvents. Methylene chloride provided a more complete extraction of phenolics than hexane or ether; a two-step extraction procedure was developed using the ion-pair reagent tetrabutylammonium chloride. Recoveries of 2-chlorophenol (2-MCP), 2,4-dichlorophenol (2,4-DCP), p-chloro-o-cresol, 2,4,6-TCP, PCP,

2-NP, 4-NP, 2,4-DMP, 2,4-dinitrophenol and 4,6-dinitro-o-cresol were greater than 90% and the recovery of PHE was 75%. Lamparski and Nestrick (99) found that benzene gave recoveries similar to those obtained with diethyl ether but extracts contained fewer interfering solutes. A number of solvent extraction systems were investigated by Bruns and Currie (104) for the removal of PCP and tetrachlorophenols (TeCPs) from carrots and potatoes. Potatoes were best extracted by blending for 5 minutes (min) with acidified acetone; carrots required a 20 hr Soxhlet extraction with the same solvent. Ethyl acetate was found to be the most suitable solvent for PCP extraction from lettuce (101) while chlorophenols were most efficiently recovered from soil with 95% ethanol (112).

In general, solvent extraction of underivatized phenols from aqueous solution is often less efficient than desired (82, 113). Recoveries of phenols and anilines from aqueous solution can be improved by the addition of neutral salts or organic reagents (114) but quantitative recoveries are rarely achieved. Murray (113) extracted ppm levels of PHE, m-CRE, p-CRE, o-CRE, 3,4-DMP and 2,6-DMP from a one-litre (L) water sample using a single 50 millilitre (mL) aliquot of chloroform; recoveries of 20%, 39%, 40%, 48%, 81% and 83%, respectively, were achieved. It was observed that more than ten sequential chloroform extractions would be required to achieve 90% recovery of PHE. Lamparski and Nestrick (99) reported that while recoveries of 60-100% were obtained by benzene extraction in the concentration

range 20-40 ng/mL, recoveries from water were poorer at lower concentration levels. Large volumes of sample and solvent must generally be used to extract a detectable amount of the compound of interest. Shackelford and Webb (87) found solvent extraction difficult to apply to very contaminated samples due to emulsion formation and recommended the use of continuous liquid extractors.

Continuous liquid extraction (CLE) has been used for the analysis of bulk water or sediment samples (84, 87, 115). Large volumes of water can be processed by CLE, but the procedure is time consuming and extraction efficiencies are usually poor. Goldberg and Weiner (84) added 5 milligrams (mg) each of a number of chloro-, nitro- and alkylphenols to 18 L of water. Following a 3 hr CLE with methylene chloride, less than 40% recoveries were achieved for PHE, 2-MCP, 4-MCP, guaiacol, o-CRE and m-CRE.

Steam distillation, the American Public Health Association standard procedure for the analysis of wastewaters (116), gave satisfactory recoveries of most, but not all phenols (115, 117). Giger and Schaffner (118) described a procedure which involved the distillation of an acidified water sample followed by an alkaline extraction to remove nonacidic compounds. 4-MCP and other highly soluble phenols such as guaiacol, NPs and PHE were not amenable to analysis by the described procedure. Mousa and Whitlock (115) recovered greater than 74% of the 2-MCP, 2-NP, 2,4-DCP, 4-chloro-m-cresol, 2,4,6-TCP and PCP added to a 500 mL

water sample at the 0.2 ppm level using steam distillation. Recovery of PHE and 4-NP were, however, only 55% and 3.1%, respectively. 4-NP forms very strong hydrogen bonds with water and cannot be recovered efficiently from aqueous solution using steam distillation.

As alternatives to recovery by distillation or solvent extraction procedures, methods which permit the adsorption of trace organic compounds from aqueous solution with porous organic polymers (119) such as polyurethane foam (55), Separon SE (120), XAD resin (121-123), N-vinyl-2-pyrrolidone polymer (124) or ion exchange resins have been developed. Adsorption methods are well suited to the recovery of trace organic compounds from large volumes of water. Sorbents are selected which have a high affinity for the compounds of interest and from which retained substances can be desorbed with minimal eluant volumes. The degree of success reported for the use of Amberlite XAD resins varies considerably. Ramstad and Armentrout (125) recovered 95% of 2,4,5-TCP adsorbed onto XAD-2 resin from aqueous solution using an alkaline methanol eluant; the performance of XAD-2 resin was found to be superior to charcoal and reverse liquid chromatography phases. XAD-2 resins were used by Hunt *et al.* (126) to isolate and concentrate phenols in petroleum wastewaters. The phenolics were desorbed from the resin with a sodium hydroxide solution and methylene chloride extraction proved to be most suitable for recovery of the phenolics from the aqueous phase after desorption from the

resin. The overall efficiency of the procedure was, however, only 25.4% for 6-chloro-o-cresol. Voss et al. (91) extracted adsorbed chlorophenols and chloroguaiacols from XAD-2 resin first using acetone and then methylene chloride and reported quantitative recoveries. Edgerton et al. (75) passed hydrolyzed urine samples through XAD-4 columns and eluted the adsorbed chlorophenols with 2-propanol in hexane. Exhaustive cleanup and extraction of the resin was necessary before use and interfering peaks originating from the resin material were difficult to eliminate completely. Recoveries from urine fortified with 0.01 to 1.00 ppm of chlorophenols averaged better than 80%. Voznakova and Popl (120) developed a method for the recovery of phenols in the 1-10^3 ppb concentration range from water by sorption onto Separon-SE, a styrene-ethylenedimethacrylate copolymer. Compounds were heat-desorbed directly onto the GLC column for analysis.

Chriswell et al. (127) determined phenols at ppb to ppm levels in natural waters and treated drinking waters by adsorption on an anion exchange resin, elution with acetone and measurement by GLC using Tenax GC or OV-17 columns. Recoveries of greater than 80% were achieved at concentrations of 0.01 to 1.7 ppm. Renberg (128) applied a similar ion exchange column procedure to the isolation of chlorophenols in tissue, soil and water. Using the strongly basic Sephadex QAE A-25 anion exchange resin, greater than 97% of 2,4,6-TCP, 2,3,4,6-TeCP and PCP added to soil at the 0.5 to 1.5 ppm level and to water at

the 0.5 to 1.5 ppb level were recovered. Recoveries from tissue extracts spiked with 0.1 to 0.3 ppm 2,4,6-TCP, 2,3,4,6-TeCP and PCP were 74%, 90% and 92%, respectively. Stalling et al. (129), however, found ion exchange chromatography unsatisfactory for processing whole fish extracts since large amounts of fatty acids co-extracted with the compounds of interest. Mousa and Whitlock (115) investigated the use of anion exchange resins for the isolation of chlorophenols from raw industrial wastes with no previous cleanup. Although the ion exchange method was promising, high concentrations of oils, tars and other industrial contaminants caused resin destruction.

Once extraction into organic solvent has been accomplished, many methods require evaporation of the resulting solution to dryness before analysis (33, 91, 96, 110, 111). It has been found (76, 111), however, that evaporation causes substantial losses of volatile phenols and produces erratic results. Solvent volumes have been reduced without solute loss using Kuderna-Danish evaporators (25, 88).

c) Cleanup

Earlier reported methods for the determination of phenols in blood, urine and water (64, 65, 130, 131) often did not include chromatographic isolation steps. Such preliminary chromatographic procedures have now become common practice as method detection sensitivities continue to improve. Column purification methods have been used both prior to derivatization

to remove interfering compounds from the sample extract and following derivatization to purify reacted phenols and anilines. Introduction of organic contaminants into samples from adsorbents can be avoided by heat treatment or Soxhlet extraction of the material prior to use (132). Silica gel (102, 107, 133), Florisil (25) and gel permeation columns (86, 105, 129, 134-136) have been used to purify sample extracts. Florisil (86, 89, 104), silica gel (62, 85, 98, 137-139) and acid alumina (72, 77, 90, 107, 133) columns are frequently used to clean sample extracts following derivatization. Edgerton and Moseman (72) concluded that cleanup of urine extracts derivatized with diazomethane using acid alumina column chromatography was essential for the determination of PCP concentrations below 30 ppb. It was reported that with column purification, as little as 1 ppb of PCP in a 2 mL urine sample could be detected as its methyl ether derivative using GLC with electron capture detection (ECD). The EPA (77) adopted the Edgerton and Moseman procedure and reported that recoveries of PCP from urine at fortification levels of 5 ppb averaged 90%. When methods without cleanup were tested, recoveries of less than 80% were achieved at fortification levels of 30 ppb or less in urine. Edgerton *et al.* (74) reported the use of acid alumina chromatography not only for cleanup of derivatized phenols, but also for the separation of these compounds into groups. Two fractions, one containing methyl ethers of 2,3,4,6-TeCP, 2,3,5,6-TeCP, PCP and pentachlorothiophenol and the other containing methyl ethers

of 2,3,4,5-TeCP, tetrachloropyrocatechol and tetrachlorohydroquinone, were eluted from an acid alumina column with 10% benzene in hexane and 40% benzene in hexane, respectively. GLC analysis of the two fractions individually allowed the resolution of methyl ethers of several closely related chlorophenols.

Lamparski et al. (107, 133) purified benzene-hexane extracts of tissue or bovine milk samples using small silica gel columns. The extracts were methylated and the derivatized samples were run through a bed of acid alumina for further purification. Using this two-column procedure, a PCP detection limit of 10-15 ppb was achieved in both animal tissue (107) and bovine milk samples (133).

Gel permeation chromatography (GPC) is often used to isolate phenolic residues from samples with high fat content and is recommended by the U.S. Food and Drug Administration for the analysis of chlorophenols in foods (86, 135). In the method described by Heikes and Griffit (86), PCP extracted from foods with methylene chloride was purified by GPC, methylated and then further purified by Florisil chromatography prior to analysis. Since GPC does not involve adsorption, quantitative recoveries might be expected. However, Dougherty and Piotrowska (105) reported only a 28% recovery of applied PCP at the 5 ppb level. Kuehl and Leonard (134) found that while the use of BioRad SX-2 (copolystyrene-2% divinylbenzene) permitted separation of PCP from Arochlor 1254 and corn oil, cyclohexane elution recovered

only 10% of polar organics such as PCP from the column. An elution mixture of methylene chloride-hexane (50-50) gave the highest recoveries and most complete purification of chlorophenols. Using this procedure, many substituted anilines, phenols and other trace organic contaminants were identified in fish tissues with recoveries of greater than 80%.

Stalling et al. (129, 136) investigated the use of alkali metal hydroxide-treated silica gels for concentration and separation of trace acidic compounds. Silica gels treated with lithium, sodium, potassium and cesium metal hydroxides were evaluated and phenolics were best retained by the cesium hydroxide columns. Acidic residues were isolated from fish tissue extracts by a sequential chromatographic process through a series of GPC, cesium silicate and carbon foam columns.

2. Identification and Quantitation

Following isolation and purification, extracted phenolic and anilino residues must be separated, identified and quantitated by an appropriate technique. GLC is the most commonly used separation technique and new column packings, capillary columns as well as derivatives have been developed for this purpose. Specific and sensitive detectors are available for GLC, including the mass spectrometer in electron impact, chemical ionization and negative chemical ionization modes. Improvements in HPLC columns and detectors have led to increased use of the technique for trace

residue analysis. Instrumental advances as well as analytical modifications to reduce interferences and increase sensitivity have improved spectrophotometric methods.

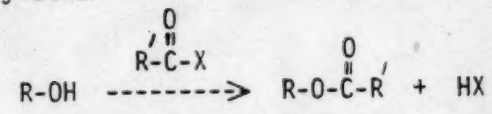
a) Gas-Liquid Chromatography

The polarity of phenols and anilines adversely affects both their extraction efficiency from aqueous solution and their chromatographic properties. GLC analysis generally involves both extraction and derivatization steps although direct aqueous injection (DAI) methods have also been developed (140-142). Acylation, alkylation, silylation and condensation reactions (Figure 1) are frequently used to derivatize primary and secondary amine groups and hydroxyl groups prior to GLC analysis. Such derivatives generally improve chromatographic behavior as well as increase sensitivity and selectivity of detection. Simple and rapid derivatization reactions using stable, pure and easily handled reagents are ideal. Reactions should proceed quantitatively or at least reproducibly and the derivatives must be easily separated from unreacted reagents and reaction products.

i. Direct Analysis

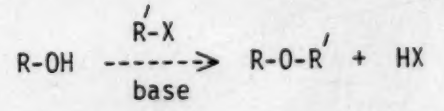
Considerable effort has recently been devoted to the development of column packings which allow the GLC analysis of underivatized phenols either by DAI or following extraction from the sample matrix. Bartle et al. (140)

1) Acylation

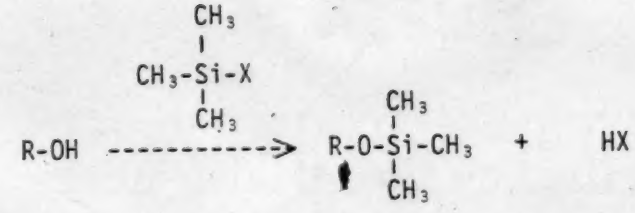


(X = halogen or good leaving group)

2) Alkylation



3) Silylation



4) Condensation

Figure 1. Four basic reactions, acylation (1), alkylation (2), silylation (3) and condensation (4) are commonly used to prepare derivatives for gas-liquid chromatography.

described the use of a Tenax GC (143) column coated with a polymetaphenyl ether OS-138 liquid phase for DAI and reasonably symmetrical peaks were obtained at the ppm level. Baker and Malo (141) evaluated various liquid phases and solid supports for DAI. Best results were obtained with FFAP, a reaction product of Carbowax 20M and 2-nitroterphthalic acid, coated on a Teflon support; phenolics could be quantitated at levels of 1-10 mg/L. Monochlorophenols, certain dichlorophenols and m- and p-cresol isomers were not resolved. Baird et al. (24, 144) used 4% dinonyl phthalate on Chromosorb G for DAI analysis of PHE, CREs, MCPs, DCPs and DMPs in wastewaters. The value of DAI methods is, however, limited by the low sensitivity which can be achieved. Interference from the water peak, appearance of memory or ghost peaks and the instability of GLC detectors in the presence of water vapor are some of the problems which hinder trace analysis by DAI. Retention times are often irreproducible and water strips the liquid phase from the packings, thus decreasing column life.

Phenols, and to a lesser extent anilines, have been analyzed gas chromatographically without derivatization following extraction from the aqueous sample matrix (145-149). Because hydrogen bonding and polar interactions cause poor peak shapes, the analysis of free phenol and aniline extracts using conventional untreated GLC column

packings is, like DAI, usually restricted to concentrated samples. Van Langeveld (103) extracted PCP from toy paints with acetone and analyzed the extracts directly using a 15% Carbowax column. The underivatized PCP eluted after 18 min at 210°C and the minimum detectable concentration was 1 ppm. Hussain and Kifayatulla (150) analyzed chlorophenols using untreated SE-30, diethylene glycol succinate (DEGS) and Carbowax 20M columns. Several chlorophenol isomers were not resolved; GLC peaks were broad and showed some tailing. The sensitivity of the method was not specified, but trace analysis at the ppb level would not be possible.

Graphitized carbon blacks (GCB) coated with FFAP (151) or PEG 20M (152) have been used for GLC of underivatized phenols and substituted anilines. Bacaloni et al. (152) described a method for preparing glass capillary columns coated with PEG 20M on GCB. The columns were operated in gas-solid, gas-liquid or gas-liquid-solid chromatographic modes, depending on the amount of stationary phase coated on the walls. Columns were made with varying liquid phase loadings and while tailing was experienced with some columns, a heavy loading of PEG 20M was well suited for the chromatography of underivatized phenols and anilines. Detection limits were not specified.

A number of investigators have described methods for deactivation of conventional column packing materials to minimize tailing. Giger and Schaffner (118) chromato-

graphed phenols without prior derivatization on glass capillary columns coated with OC-73 and deactivated by persilylation. White and Parsley (153) found that injection of 2,4-dichlorophenoxyacetic acid on Carbowax 20M enhanced the sensitivity and reproducibility of the column and allowed the analysis of underivatized chlorophenols and chlorocresols in picogram amounts. Bhattacharjee and Bhaumik (154, 155) demonstrated that rubidium benzenesulphonate modified with Carbowax 20M and ascorbic acid was an excellent stationary phase for the separation of cresol and dimethylphenol isomers. When an Apiezon L column, similarly modified, was used in conjunction with the rubidium column, complex mixtures of alkylphenols could be identified. The two columns were complimentary; compounds overlapping on one column were completely resolved on the other. Phosphoric acid (H_3PO_4), the most common deactivation agent, has been used to treat columns coated with DEGS (95, 156) , Carbowax 20M (157), GCB (158) and SP-1000 (106). Ress and Higginbotham (159) demonstrated that a variety of polar stationary phases including DEGS, Carbowax 20M, ethylene glycol succinate, ethylene glycol malonate and ethylene glycol adipate could be used for GLC of underivatized phenols following treatment with 2% H_3PO_4. Barthel *et al.* (95) observed that PCP could not be chromatographed on a phosphoric acid-free DEGS column and peak tailing was still observed following a 1% acid treatment.

Symmetrical peaks were obtained using a 2% H_3PO_4-treated DEGS column; an injection of 0.02 ng of PCP could be detected. A number of H_3PO_4-treated column packings are commercially available, including SP-1240 DA (160), SP-1200 and OS-138 (161). Shackelford and Webb (87) compared derivatization methods to direct GLC procedures using deactivated columns. For initial quantitative analysis of unknown samples, they advocated GLC of underivatized phenols on SP-1240 DA. Acid treatment reduces tailing of free phenols, however, H_3PO_4 is thermally unstable above temperatures of 180°C and is neutralized by basic materials resulting in rapid column deterioration (158, 160). Acid-treated columns are not suitable for the analysis of samples containing impurities which can only be eliminated from the column by high temperature baking.

Shackelford and Webb (87) compared the performances of five stationary phases for the GLC analysis of underivatized phenols. Packings other than SP-1240 DA and Tenax GC, including SP-1000, Ultra-bond and SP-2250, proved unsuitable for direct chromatography of PCP. 2,4-Dinitrophenol and 4,6-dinitro-o-cresol did not elute from SP-2250, SP-1000 or Ultra-bond columns even at the 1000 ng level. All five stationary phases gave poor results for the GLC analysis of 4-NP at injected concentrations of less than 100 ng. Column stability as well as the resolution efficiency of SP-1240 DA were considered superior to Tenax

GC. Temperature programming caused the Tenax packing to harden to an impenetrable plug. If the Tenax column was maintained at temperatures above 150°C without intermittent cooling to room temperature, column life could be prolonged. Gebefugi et al. (8) reported that GLC properties of several similarly prepared Tenax GC columns were not comparable. Van Roosmalen et al. (73) used a Tenax GC column to analyze gas chromatographically PHE, 2-MCP, 2,4-DCP, 2,6-DCP, 2,4,6-TCP and 2,3,5,6-TeCP following a modified Soxhlet extraction of 50 mL urine samples obtained from occupationally exposed workers. Detection limits ranged from 0.1 mg/L for PHE to 1 mg/L for the di- and trichlorophenols. The EPA initially advocated the use of Tenax GC for the GLC analysis of phenols; peak tailing was, however, observed and most nitrophenols were adsorbed at low concentrations (160). SP-1240 DA (162) has now replaced Tenax GC (88) as the column packing recommended by the EPA for the analysis of phenols. The high thermal stability of Tenax GC is still used to advantage for the analysis of high-boiling alkylphenols (163).

The analysis of ppb levels of free chlorophenols using conventionally coated polyester packings often leads to problems of column instability, liquid stationary phase bleed and loss of column efficiency and sensitivity. Edgerton and Moseman (164) outlined a procedure for the

preparation of support bonded polyester column packings. Using a double bonded DEGS column, chromatography of free chlorinated phenols at subnanogram levels was possible. Edgerton et al. (75) used double support bonded DEGS and support bonded butane-1,4-diol succinate for the analysis of underivatized chlorophenols extracted from human urine. The detection sensitivity for DCPs and TCPs (except 3,4-DCP, 3,5-DCP and 3,4,5-TCP) was 1 ppb and for the TeCPs and PCP was 2 ppb. The GLC response for the meta-substituted phenols was relatively poor; sensitivities for 3,4-DCP, 3,5-DCP and 3,4,5-TCP were 15 ppb, 50 ppb and 50 ppb, respectively. Lamparski et al. (165) described the use of a bonded DEGS column for the analysis of underivatized PCP extracted from preserved wood. Lamparski and Nestrick (99) also used a bonded nitro-DEGS stationary phase to analyze a series of chlorophenols after derivatization with heptafluorobutyric anhydride (HFBA). Several high efficiency low-bleed bonded stationary phases were compared with nonbonded phases for the analysis of phenyl heptafluorobutyryl derivatives. The bonded phases possessed longer column stability, and improved efficiency and sensitivity; column bleed was minimal and lower operating temperatures were possible.

ii. Alkyl Derivatives

The best means of overcoming GLC peak tailing involves

the preparation and analysis of less polar derivatives. Alkyl ethers of phenols are commonly prepared for GLC analysis. Derivatization procedures included in the Bevenue and Beckman review (166) of PCP analysis all involved the use of diazoalkyl reagents. The diazomethane derivatization procedure first introduced by Bevenue et al. (64) for the analysis of PCP in urine is shown in Scheme 2.

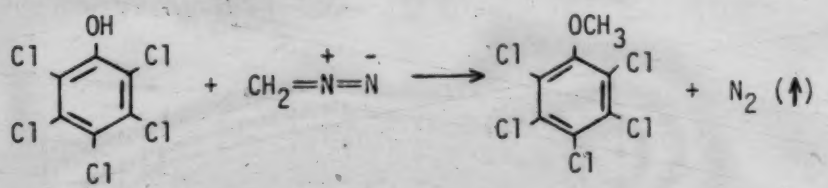
Urine sample (25 mL)

↓ Acidify with concentrated H_2SO_4

Extract PCP twice with 15 mL aliquots of petroleum ether

↓ Evaporate solvent to dryness

Alkylate residue with diazomethane:



PCP diazomethane methyl ether derivative

Scheme 2.

The original procedure has been modified (63, 72, 74, 131) and is still widely used (65, 77, 100). The EPA method

(77) for analysis of chlorophenols is based on the procedures described by Edgerton et al. (74) and Edgerton and Moseman (72). PCP as well as chlorinated metabolites of PCP and hexachlorobenzene in urine are analyzed by ECD-GLC following hydrolysis, benzene extraction and derivatization with diazomethane. Recoveries of pentachlorothiophenol, tetrachloropyrocatechol and tetrachlorohydroquinone become low and erratic if the analytical procedure is interrupted before the methylation step.

The Bevenue method for the analysis of urinary PCP has also been modified to overcome specific problems associated with analysis of other biological fluids (130, 131, 167, 168). The method presently recommended by the EPA (169) for serum analysis incorporates portions of the methods described by Rivers (131) and Cranmer and Freal (63). The procedure consists of a 2 hr extraction of the acidified sample with benzene, followed by methylation with diazomethane and analysis by ECD-GLC. Recoveries from serum samples fortified with PCP were over 90% at concentrations of 190 ppb or higher. The lower limit of detection was 10 ppb. Hoben et al. (170) reduced the serum sample size required and incorporated a Florisil cleanup step. Hexane was used as the extraction solvent because its toxicity is lower than that of benzene and the extraction efficiencies of the two solvents were comparable. In areas such as Dade County, Florida, where the general population is

continuously exposed to PCP, blood serum levels in excess of 100 ppb are not uncommon (85, 169). Morgade et al. (85) analyzed fifty-eight serum samples for 2,4-DCP, 2,3,5-TCP, 2,4,5-TCP, 2,4,6-TCP, 2,3,4,5-TeCP, 2,3,4,6-TeCP, 2,5-dichloro-4-bromophenol and PCP. Using methylene chloride extraction followed by diazoethane derivatization and ECD-GLC analysis, only PCP was detected in the serum samples and in samples of drinking water. PCP levels in water ranged from <30 to 340 ppb and serum samples contained 10-120 ppb. Klemmer et al. (171) reported that despite mean serum levels of 3.78 ppm PCP in twenty-two chronically exposed workers, no clinical abnormalities were apparent.

Diazoalkanes have been used to derivatize many different phenols extracted from a variety of sample matrices. GLC analysis of pentachloroanisole, prepared from pentachlorophenol by methylation with diazomethane, is the method currently used by the U.S. Food and Drug Administration for the detection of PCP residues in foods (86, 135). Although diazomethane (53, 74, 79, 86, 87, 90, 97, 100, 107, 128, 133, 170-175) and diazoethane (85, 89, 98, 104, 139, 170, 176, 177) are most commonly used, other diazoalkyl reagents such as amyl (89), propyl, isobutyl, butyl, hexyl and isoamyl compounds (63, 65, 138) have been investigated. Faas and Moore (89) reported that 0.002 ppb PCP in sea water could be determined by ECD-GLC analysis of

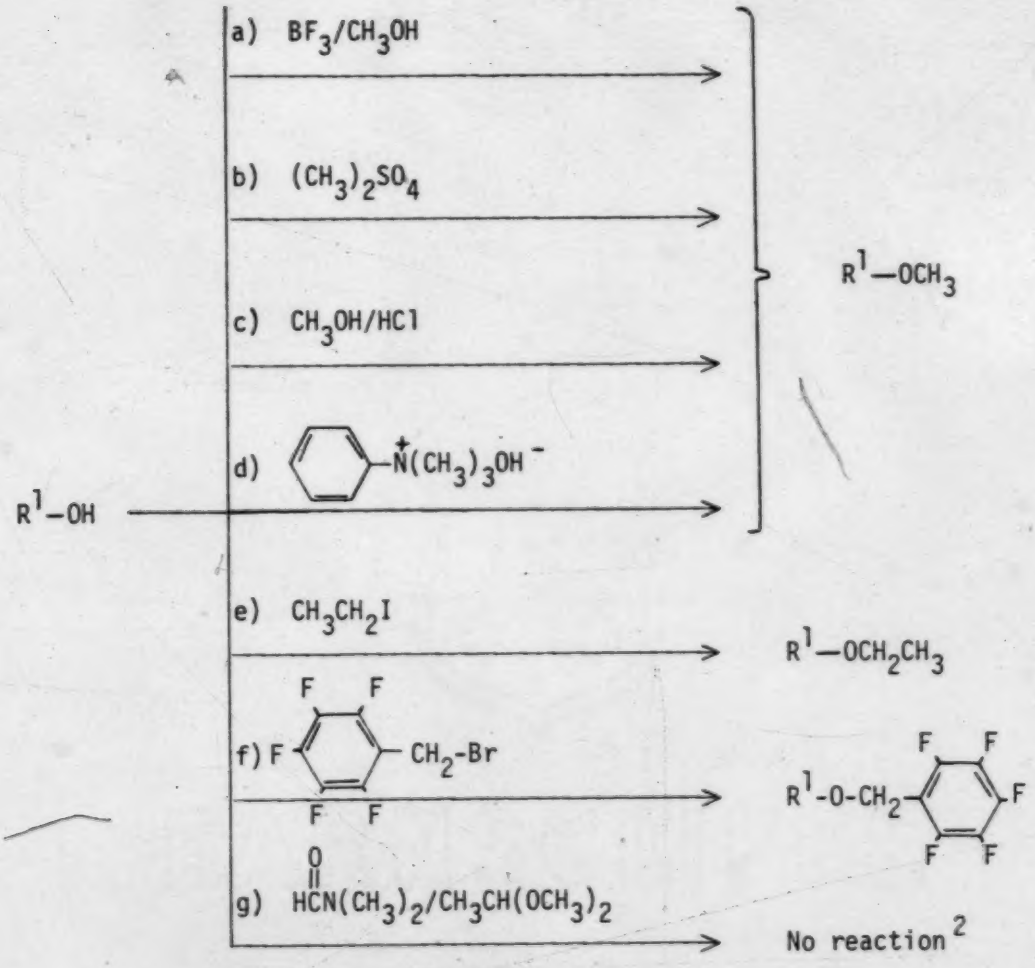
amyl derivatives; 0.01 ppm PCP could be detected in tissues as its ethyl ether. Tulp and Hutzinger (172) prepared deuteriomethyl derivatives of hydroxychlorobiphenyls by reaction with deuteriodiazomethane. A mixture of derivatives was obtained and the advantage of deuteration for analysis by mass spectrometry was lost. The preparation of several different alkyl ether derivatives of the same sample can confirm the suspected identity of phenols. The use of alkylating reagents other than methylating reagents is also advantageous since methoxy derivatives methylated by natural metabolic processes exist (33, 172, 178).

Lindstrom and Nordin (33) successfully used diazoethane to analyze chlorophenols as ethyl ethers in spent kraft pulp mill bleach liquors. Shackelford and Webb (87), however, found that while the conversion of simple phenols to anisoles was nearly quantitative using single pure standards, poor results were obtained for mixtures or spiked industrial effluent samples. When PHE, which required the longest reaction period, had been quantitatively derivatized, decomposition of other anisoles had already begun. Complex chromatograms, with several peaks for each phenol, were obtained. Hopps (179) reported that diazomethane did not react quantitatively with all phenols and that undesirable side reactions occurred. Although PCP reacts quantitatively and other chlorophenols are converted

to methyl ethers with diazomethane, Webb et al. (25) found that guaiacol, vanillin and other phenols in kraft paper mill wastes gave poor yields.

Diazoalkane reagents are extremely toxic and potentially explosive. Other alkylating reagents (Figure 2), including boron trifluoride/methanol (102), methyl iodide (79, 172, 178), ethyl iodide (172), dimethyl sulfate (180) and pentafluorobenzyl bromide (87, 181-184), have been used as alternatives. Wakimoto et al. (180) methylated PCP with dimethyl sulfate and reported GLC detection limits of 0.01 ppb in water and 1.0 ppb in soil. Webb et al. (25) compared the effectiveness of five methylating reagents, dimethyl sulfate, diazomethane, methanolic HCl, Methyl-8 and MethElute, for the derivatization of the acid fractions of kraft paper mill effluents. Methyl-8 (dimethylformamide dimethyl acetal) did not methylate phenols. Dimethyl sulfate gave much better yields of phenolic methyl ethers than did diazomethane. The reactivity of methanolic HCl for phenol derivatization was comparable to that of diazomethane; the acidic methanol did not react with vanillin or guaiacol. MethElute, (trimethylanilinium hydroxide), an "on-column" methylating reagent, also gave results comparable to diazomethane.

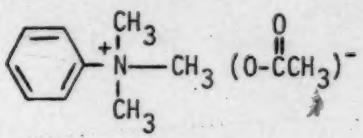
Butte (185) introduced three new methylating reagents (Scheme 3), trimethylanilinium acetate (TMAA), trimethylselenium hydroxide (TMSH) and trimethylselenium acetate



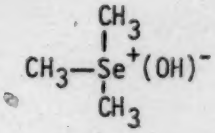
1. phenyl or substituted phenyl ring
2. Webb et al., reference 25.

Figure 2. Some commonly-used alkylating reagents for the derivatization of phenolic hydroxyl groups: a) BF_3/methanol, b) dimethyl sulfate, c) methanolic HCl, d) MethElute, e) ethyl iodide, f) pentafluorobenzyl bromide, g) Methyl-8.

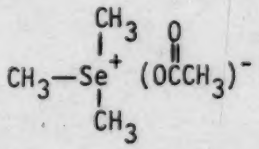
Methylating reagents:



TMAA

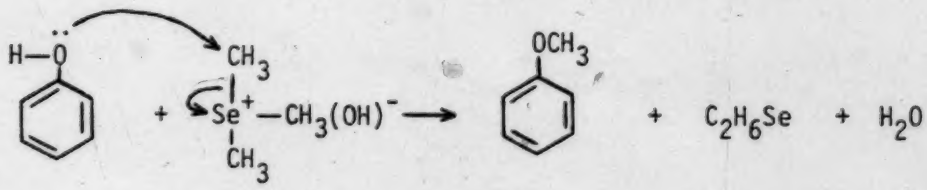


TMSH



TMSA

Reaction mechanism for "on-column" methylation of phenol with TMSH:



Phenol TMSH Anisole

Scheme 3.

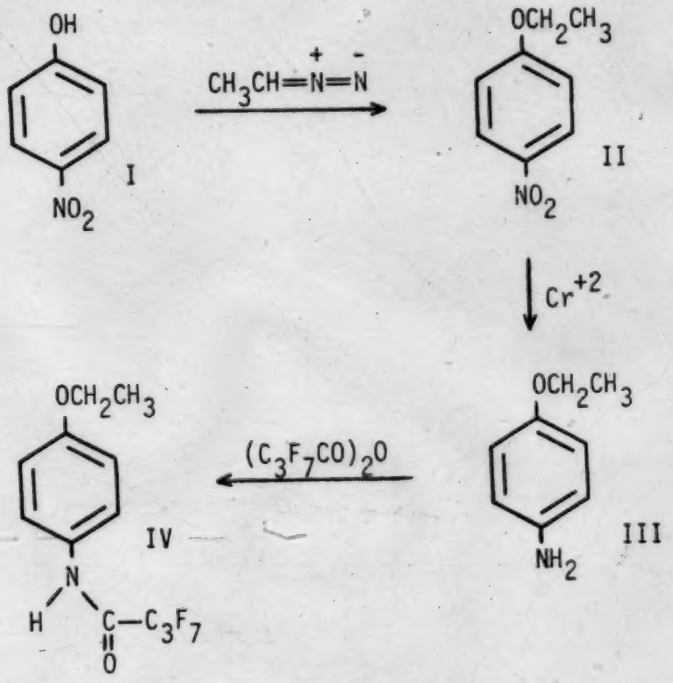
(TMSA), for use in "on-column" flash methylation procedures. TMSH proved to be a more powerful pyrolytic methylation reagent than either TMAA or TMSA. For PHE, 2,3-DCP, 3,4,5-TCP, 2,3,4,5-TeCP, PCP and 2,3,5-trimethylphenol, methylation with TMSH was almost quantitative whereas yields of 10-99% were obtained with TMAA or TMSA.

iii. Acyl Derivatives

Acyl derivatives are most commonly used for GLC of amines. N-acyl derivatives tend to be more stable than

O-acyl derivatives (186). Although diazoalkane reagents are still by far most frequently used for phenol derivatization, there has been increased interest in the preparation of acyl derivatives. Acid anhydride or acid chloride reagents are less toxic than diazoalkanes and react rapidly and quantitatively with most phenols and amines (186).

Phenols have been analyzed commonly as acetyl and trifluoroacetyl derivatives as well as pentafluoropropionyl (187), heptafluorobutyryl (187, 188), benzoyl (189), propionyl (76), chloroacetyl (8, 190) and dichloroacetyl (191) derivatives prepared using the corresponding anhydride or acid chloride reagents. Lamparski and Nestrick (99) investigated the use of heptafluorobutyryl-imidazole (HFBI) for derivatization of phenols. While derivatives of PHE, MCPs and alkylphenols showed no evidence of decomposition after storage at 4°C for 72 hr, the heptafluorobutyrates of more highly halogenated phenols were much less stable. Trichlorophenols did not react well with HFBI because of their increased acidity. Similarly, amide derivatives of amines have been prepared with many different reagents with varying degrees of success (137). Of the many reagents tested by Bradway and Shafik (137), pentafluoropropionic anhydride (PFPA) was considered best. Kirby et al. (192) reported a novel procedure for the detection of 4-NP in human urine (Scheme 4).



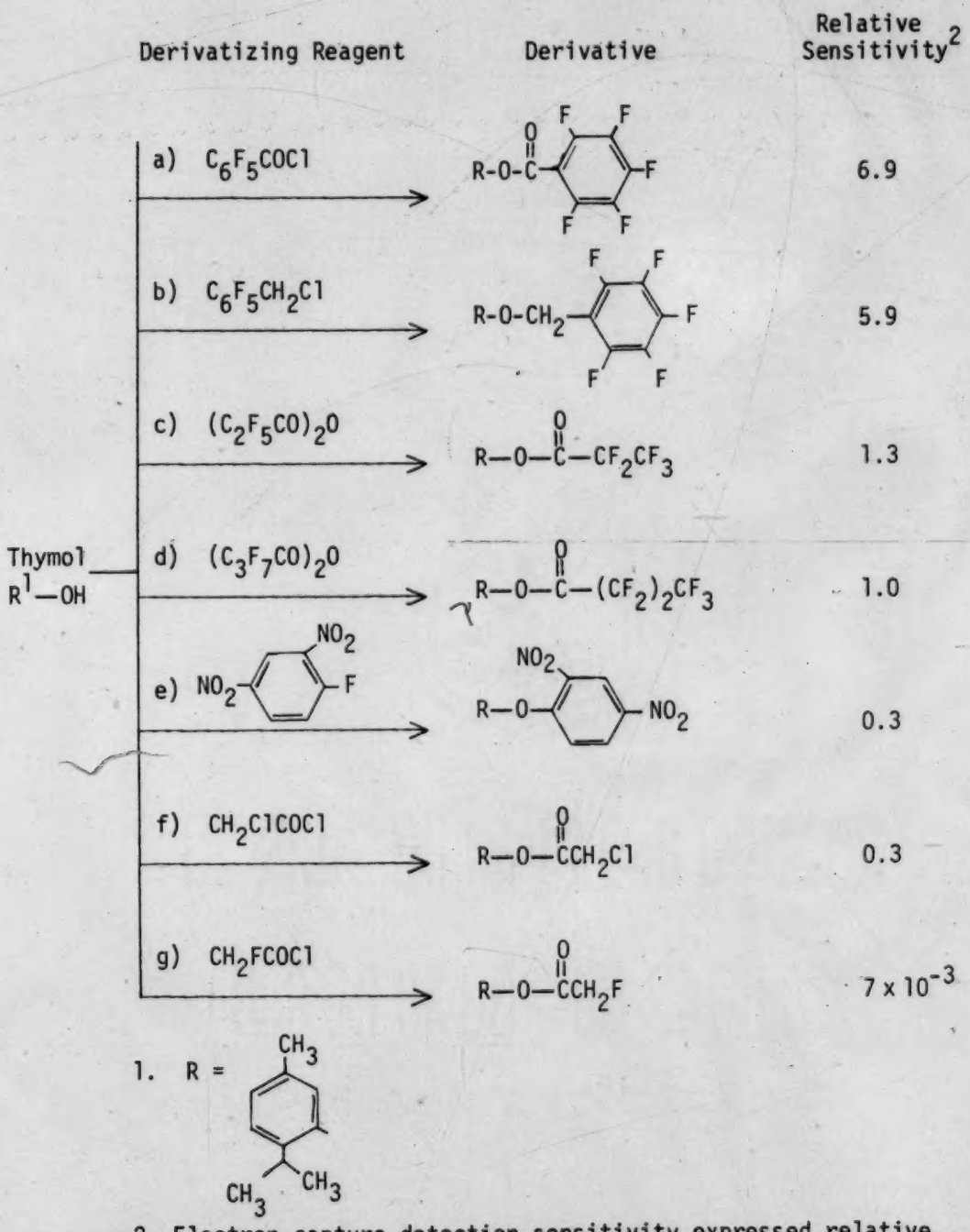
Scheme 4.

The ethyl ether of 4-NP (I) was prepared using diazoethane as described by Shafik et al. (62). For confirmation of identity, the p-ethoxynitrobenzene (II) was then reduced with chromous chloride and the resulting p-phenetidine (III) was converted to the amide (IV) by reaction with HFBA. 4-NP was identified in human urine at concentrations of 12-26 ng/mL and 18-67 ng/mL in the general U.S.A. population and parathion-exposed subjects, respectively.

Generally, the ECD response to acyl derivatives is higher than that of the corresponding alkyl derivatives (193). Using thymol as a model compound, McCallum and Armstrong (194) compared the ECD sensitivities of ester

derivatives prepared using monofluoroacetyl chloride, monochloroacetyl chloride, PFPA, HFBA and pentafluorobenzoyl chloride (PFBC) with those of ether derivatives prepared using pentafluorobenzyl bromide and 2,4-dinitro-1-fluorobenzene (Figure 3). Pentafluoroaryl derivatives provided the greatest detection sensitivity and the pentafluorobenzoate was superior to the pentafluorobenzyl ether. The detection limit for the thymol pentafluorobenzoate was 1 picogram (pg).

Acetic anhydride is commonly used for derivatization of phenols in extracts of plant and animal tissues (30, 101, 195), milk (93), biological fluids (76), and natural waters (30, 78, 196, 197). The acetate esters are prepared quantitatively and have excellent GLC properties (30). The method reported by Rudling (30) for the acetylation of PCP with acetic anhydride involved several sequential extraction steps followed by derivatization. Similarly, a number of other acetylation methods require preliminary extraction of the phenol from an acidified sample matrix into ethyl acetate (101), hexane (30, 195), benzene (8, 93, 196) or toluene (78, 80) followed by a back extraction into basic aqueous solution. The chlorophenols in the basic solution are then acetylated, re-extracted into organic solvent and analyzed gas chromatographically. Wegman and Hofstee (78) used a modification of the acetylation method described by Krijgsman and Van de Kamp (80) for



2. Electron capture detection sensitivity expressed relative to that of thymol heptafluorobutyrate.

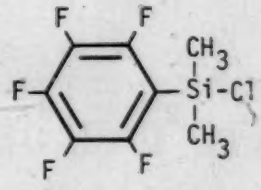
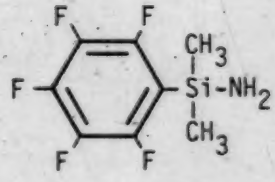
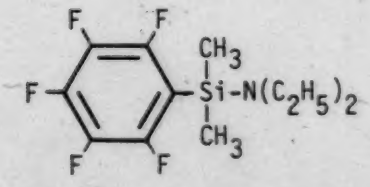
Figure 3. Thymol derivatives and their relative electron capture detection sensitivity. Derivatization reagents: a) pentafluorobenzoyl chloride, b) pentafluorobenzyl chloride, c) pentafluoropropionic anhydride, d) heptafluorobutyric anhydride, e) 2,4-dinitro-1-fluorobenzene, f) monochloroacetyl chloride, g) monofluoroacetyl chloride.

determination of chlorophenols in surface water samples in the Netherlands. The practical limits of detectability of the acetate derivatives of MCPs, DCPs, TCPs, TeCPs and PCP by capillary GLC with ECD were 2 micrograms (µg)/L, 0.05 µg/L, 0.02 µg/L, 0.01 µg/L and 0.01 µg/L, respectively. Greater than 85% recovery of chlorophenols was achieved using the back extraction method ; with capillary GLC nineteen chlorophenyl acetates including three monochlorophenols, six dichlorophenols, six trichlorophenols, three tetrachlorophenols and PCP could be resolved. The chlorophenols found most frequently and in highest concentration were 2,6-DCP, 2,4,5-TCP, 2,4,6-TCP, 2,3,4,6-TeCP and PCP. The major disadvantage of all of the acetylation reactions so far described is that the derivatization is performed following a complex series of extraction and back extraction steps.

iv. Other Derivatives

In addition to alkyl ether formation and acylation, a number of other methods have been reported for the derivatization of phenols and anilines prior to GLC analysis. Table III lists a few of the many reagents which have been used for the preparation of silyl derivatives of phenolic hydroxyl groups (84, 102, 108, 113, 120, 198-200). Amines are less frequently analyzed gas chromatographically following silylation since the N-trimethylsilyl derivatives

Table III. Silylating Reagents Used to Derivatize Phenolic Compounds Prior to Gas Chromatographic Analysis

Reagent	Common Abbreviation	Structure
N,O-Bis(trimethylsilyl)-acetamide	BSA	$(CH_3)_3Si\text{-}O\text{-}C(CH_3)=N\text{-}Si(CH_3)_3$
N,O-Bis(trimethylsilyl)-trifluoroacetamide	BSTFA	$(CH_3)_3Si\text{-}O\text{-}C(CF_3)=N\text{-}Si(CH_3)_3$
Hexamethyldisilazane	HMDS	$(CH_3)_3Si\text{-}NH\text{-}Si(CH_3)_3$
Trimethylchlorosilane	TMCS	$(CH_3)_3Si\text{-}Cl$
N-Methyl-N-trimethylsilyl heptafluorobutyramide	-	$C_3F_7\text{-}C(=O)\text{-}N(CH_3)\text{-}Si(CH_3)_3$
Pentafluorophenyl-dimethylsilyl chloride	flophemesyl chloride	
Pentafluorophenyl-dimethylsilylamine	flophemesylamine	
Pentafluorophenyl-dimethylsilyldiethylamine	flophemesyl-diethylamine	

are much less stable than the O-trimethylsilyl derivatives (186). Stalling and Hogan (198) formed trimethylsilyl (TMS) derivatives of chlorophenols by reacting them with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) in acetonitrile at 60°C for 15 min. Silyl derivatives of phenols have also been prepared using hexamethyldisilazane (HMDS) (84), N,O-bis(trimethylsilyl)acetamide (BSA) (102, 120), trimethylchlorosilane (TMCS) (174) or combinations of these reagents. Tri-Sil (113, 200), a commercially available solvent-reagent-catalyst formulation containing pyridine, HMDS and TMCS has, for example, been used to silylate phenolic hydroxyl groups.

TMS derivatives have excellent GLC properties and the mass spectra of these compounds commonly contain abundant m/z (M-15) or m/z 73 ions. Both fragment ions are consistently produced and are suitable for detection by selected ion monitoring mass spectrometry. Stalling and Hogan (198) identified compounds containing a chlorine atom ortho to the O-TMS group by the ratio of m/z 93 to m/z (M-15) fragments. Bjorseth et al. (108) reported an "on-column" method for the silylation of phenols using BSTFA. Underivatized sample extracts in butyl acetate were injected on the GLC column followed immediately by an injection of BSTFA. The efficiency of the "on-column" silylation reaction was not reported. Matsumoto et al. (102) analyzed the phenols in extracts from two urban

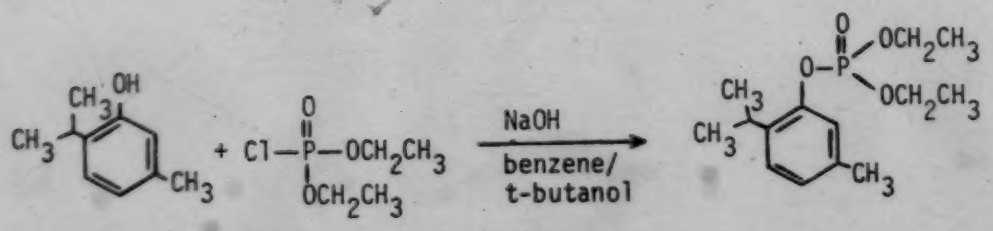
rivers in Tokyo following a two-step derivatization procedure. Phenols, extracted into ethyl acetate from acidified aqueous solution, were first methylated with boron trifluoride/methanol, hydrolyzed to free phenols with base and then silylated with BSA. Radmacher (201) developed a new silylating reagent, N-methyl-N-trimethylsilyl heptafluorobutyramide. This reagent and the pentafluoropropionamide and pentafluorobenzamide analogues contained more fluorine atoms than BSTFA and reduced silica fouling of flame ionization detectors.

Both phenols and amines can be detected gas chromatographically in the picogram and femtogram range following derivatization with the pentafluorophenyldimethylsilyl reagents flophemesyl chloride, flophemesylamine and flophemesyldiethylamine (202, 203). As is the case with other silyl derivatives, the presence of the flophemesyl group influences the fragmentation of the derivatives and strong silicon-containing diagnostic fragments are formed which are suitable for single and selected ion monitoring. The flophemesyl derivatives have good chromatographic properties, excellent thermal stability and high sensitivity to ECD. Derivatization is rapid and quantitative. Like TMS ethers, however, the flophemesyl compounds are very sensitive to hydrolysis.

Dinitrophenyl and trifluoromethyldinitrophenyl derivatives have been prepared for ECD-GLC analysis of

anilines (41, 204) and phenols (205-209). These derivatives, usually prepared by reaction with the appropriate halide reagent in the presence of base, have strong electron-capturing properties which compare favorably with derivatives prepared by reaction with chloroacetic anhydride or pentafluorobenzyl bromide (205). Farrington and Munday (206) extracted TCPs, TeCPs and PCP from chicken flesh and prepared 2,4-dinitrophenyl (DNP) derivatives by reaction with 2,4-dinitro-1-fluorobenzene in the presence of pyridine as a catalyst. Using this procedure, recoveries of 85-92% were obtained for 2,3,4,6-TeCP and PCP added to tissue samples at 0.04-0.4 mg/kg levels. Cohen et al. (205) compared several methods for the preparation of DNP derivatives of phenols using 2,4-dinitro-1-fluorobenzene but, even under the most rigorous conditions, yields of many of the phenols tested were poor. Seiber et al. (207) prepared derivatives of phenols by reaction with 4-chloro-α,α,α-trifluoro-3,5-dinitrotoluene. Using the described procedure, phenol levels below 2.5 ppb could be detected by ECD-GLC. The 2,6-dinitro-4-trifluoromethylphenyl derivatives were compared with DNP and pentafluorobenzyl ethers. The three types of derivatives were found to be similar in terms of preparation and stability. The ECD responses were highest for the pentafluorobenzyl derivatives.

Heenan and McCallum (210) monitored phenols using GLC with a flame photometric detector (FPD) following derivatization with diethyl chlorophosphate as shown in Scheme 5.



Thymol

diethyl chlorophosphate

Phosphorylated derivative

Scheme 5.

While many phenols including thymol, isoeuginol, 4-NP, 2-NAP and 2-methoxy-4-methylphenol reacted readily, hindered phenols such as vanillin and 2,4,6-tritertiary-butylphenol could not be analyzed using this method. The ECD analysis of phenols as pentafluorobenzoate derivatives was five times more sensitive than FPD analysis of the corresponding phosphorylated derivatives.

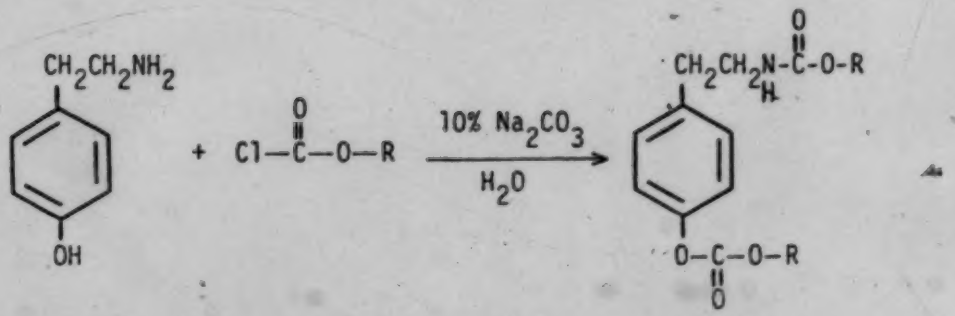
Phenols (28) and anilines (109, 211) have both been analyzed by ECD-GLC following reaction with bromine. Wegman and de Korte (109) detected ANI, 2-, 3- and 4-chloroaniline (CA) and 3,4-dichloroaniline in Rhine River water at concentrations from 0.15 to 3.0 µg/L. The minimum

detectable concentrations of anilines in surface waters following extraction and bromination were 5-15 ng/L.

The major metabolites of polychlorinated biphenyls (PCBs) are hydroxylated compounds. Tulp and Hutzinger (172) characterized these metabolites in their studies of the toxic effects of PCBs. Cyclic n-butylboronates of ortho-chlorobiphenyldiols were prepared using n-butyl boronic acid in dimethylformamide and 2,2-dimethoxypropane. Cyclic derivatives of 2,5-dichloro-2',3'-biphenyldiols and 2,5-dichloro-3',4'-biphenyldiols were prepared. The synthesis of the n-butyl boronates unambiguously distinguished ortho-chlorobiphenyldiols from other isomers.

In addition to acylation procedures, other methods for the direct derivatization of phenols in aqueous solution have been reported. Makita et al. (212) and Yamamoto et al. (213) found that alkyl chloroformates react readily with phenolic hydroxyl and amine groups directly in alkaline aqueous medium to produce the corresponding O- and N-alkyloxycarbonyl derivatives as shown in Scheme 6.

O-Isobutyloxycarbonyl derivatives of eighteen representative alkyl-, halo- and nitrophenols were stable at room temperature for up to one week and recoveries were almost quantitative. Makita et al. (212) applied the method to the analysis of PHE and p-CRE in urine. Yamamoto et al. (213) prepared derivatives of twelve amines using methyl, ethyl, isobutyl, n-butyl and n-amyl chloroformates



Tyramine

N,O-alkyloxycarbonyl derivative

R = methyl, ethyl, isobutyl, n-butyl, n-amyl

Scheme 6.

and concluded that the ethoxycarbonyl derivatives were most suitable for GLC.

b) Mass Spectrometry

Samples analyzed for trace levels of anilines and phenols are often complex mixtures containing high concentrations of industrial or natural organic compounds. With such samples, GLC retention times cannot be used for unequivocal identification because many components in the samples may possess coincident retention times. In such instances, mass spectrometry (MS) can

provide both specific and sensitive detection. While a number of other techniques, such as p-value determination (77) or ultraviolet (UV) photodecomposition (93) are available, chemical ionization (CI), negative chemical ionization (NCI) or electron impact (EI) MS are most commonly used to confirm the suspected identity of organic components (25, 214).

MS has been used not only for secondary confirmation but also for primary detection and quantitation. Freudenthal (215) described a method which used MS as an element-specific detector for various halogenated compounds in Rhine River water; the detection limit for such compounds was 1 pg. Ingram et al. (175) described a mass spectrometric isotope dilution technique for the determination of PCP in water. Following derivatization with diazomethane, PCP was determined from the ion intensities in the mass range m/z 278-290. Matsumoto et al. (102) analyzed PCP in river water by EI-MS with a detection limit of 10 ng/L.

Selected ion monitoring mass spectrometry (SIM-MS) involves the use of the mass spectrometer as a very specific detector set to monitor one or more fragment ions which are characteristic of the compounds under investigation. Using MS and SIM-MS, Kunte and Slemrova (197) identified and quantitated forty-three different phenolic substances in extracts from the Rhine and Main Rivers. The major phenolic components detected were substituted phenols with one or more alkyl-, chloro- or nitro-group; all concentrations were below 1 µg/L. Acetate

derivatives, prepared in a back extraction procedure, were suitable for both EI-MS and SIM-MS. Wu et al. (178) developed a SIM program to detect PCP in fish tissues. The methyl ether derivative of PCP in tissue extracts was prepared using methyl iodide. Two diagnostic fragment ions of the pentachloroanisole derivative (m/z 280, m/z 237) and the internal standard pentachlorophenetole (m/z 294, m/z 237) were monitored. Precision and linearity of the SIM method gradually deteriorated as the GLC column and ion source became contaminated with other sample impurities. When the column was well conditioned, 10 pg of PCP could be detected as its methyl ether but after several injections PCP adsorption resulted in loss of sensitivity. Bose and Fujiwara (189) used CI-MS for SIM analysis of PCP in crab tissue extracts derivatized with benzoyl chloride. The pentadeuterated benzoate of PCP was used as the SIM internal standard.

NCI-MS, like SIM-MS, provides a very specific GLC detector system which is used to screen samples for organochlorine residues in the presence of interfering material (105, 117, 216, 217). The technique has been used to examine PCP formulations as well as biological and environmental samples. Dougherty and Piotrowska (105) used NCI-MS for the analysis of PCP in lipid-rich foods such as candy bars and beef fat. Semiquantitative results were obtained using p-chlorobenzophenone as the internal standard. Kuehl and Dougherty (117) found PCP in human seminal fluid, human adipose tissue and fish tissue samples examined by NCI-MS.

c) High Performance Liquid Chromatography

In their 1967 review of PCP analytical methods, it was notable that Bevenue and Beckman did not include high performance liquid chromatography techniques. Since then, however, HPLC has become a fast, reproducible and sensitive analytical technique for trace organic analysis. Hussain and Kifayatulla (150) reported conditions for the separation of underivatized chlorophenols and chlorocresols using both GLC and HPLC. Certain compounds which could not be separated by GLC were resolved by HPLC.

A minimal sample cleanup requirement is often considered to be one of the major advantages of HPLC over GLC techniques. The degree and type of sample preparation required in HPLC depends, however, on the nature of the sample and the analytical sensitivity required. Little sample preparation is necessary for the HPLC analysis of high phenol and aniline concentrations (94, 218). Daniels and Swan (94) developed an HPLC method for the analysis of TeCP and PCP on lumber surfaces which permitted the processing of one hundred and forty-four samples in 48 hr. Hayes (219) used a paired-ion reverse phase HPLC method to analyze PCP formulations. Formulations, containing between 1.81 to 3.12% PCP, were dissolved in solvent and run directly on the HPLC system. The linear concentration range for the direct analysis of PCP was 0.5 to 5.5 mg/mL.

In general, methods for the analysis of trace quantities of phenols and anilines in foods (220), biological samples (89,

221) and water (81, 83, 110, 111, 222, 223) still demand some form of sample cleanup such as conventional solvent extraction or pre-column trace enrichment. The degree and type of sample purification performed depends on the nature of the sample, the number of compounds which must be simultaneously determined and the detection sensitivity required. To achieve comparable sensitivity, Lawrence (224) indicated that HPLC with UV detection required at least as much sample extraction and purification as ECD-GLC.

For HPLC analysis, trace organic compounds have been concentrated by simple organic solvent extraction methods (81, 96) or a combination of ion-pair and solvent extraction (83, 110, 111), as described earlier. Trace enrichment is an alternative method for concentration of organics from aqueous solution (225, 226). After pumping a 20 mL raw river water sample onto a pre-column, Mayer and Shoup (223) flushed the solute through the short enrichment column onto a reverse phase analytical column with mobile phase solvent. While one sample was being chromatographed another could be enriched. Edwards et al. (227) adsorbed trace organics in industrial waste effluents onto a Bondapak C_{18}/Porasil B nonpolar bonded phase pre-column prior to chromatographic analysis.

Smit et al. (228) described a unique instrumental modification which allowed the analysis of trace concentrations of PCP and eleven other chlorophenols in aqueous solution without sample preconcentration. Correlation chromatography

substantially increased detector sensitivity since system noise was not cross correlated with input. Using a UV detector at a wavelength of 254 nanometers (nm), 6 µg/L PCP could be analyzed directly in the water sample without extraction steps.

Various HPLC columns and eluants have been used to separate mixtures of phenols and anilines. Reverse phase systems (40, 83, 94, 110, 111, 219, 222, 223, 227-234) are commonly used; conditions for normal phase HPLC (150) have also been developed. In reverse phase chromatography the presence of acetic acid in the mobile phase gradients (111) prevented phenol peak tailing. Stalling et al. (129) compared the performance of columns containing silica gel, cesium silicate and LiChromosorb RP-18 for the HPLC separation of twelve phenols. Hayes (219) described the use of reverse phase paired-ion chromatography for the analysis of PCP in formulations. A tetrabutylammonium counter ion was used in the mobile phase to complex ionized phenols. Armentrout et al. (229) increased selectivity for phenols by using a polymeric cation exchange resin column. Aromatic amines, also detected by the electrochemical detector, did not elute from the reverse phase column with the acidic acetonitrile-water eluant. Olsson et al. (235) described the use of polyvinylpyrrolidone and anion exchangers in the acetate form for HPLC of a large number of chlorinated phenols. Acetic acid (3-7 M) was used as the eluant for both columns. Sakurai and Ogawa (236) separated ANI and 2-, 3- and 4-AP using cation exchange HPLC. The chromatographic procedure was lengthy and

ANI, which had the longest retention time, gave a broad peak. Sternson and DeWitte (232) described a reverse phase HPLC system for the analysis of aromatic amines using an aqueous mobile phase containing nickel. 2-AP formed a stable chelate with the metal and could be resolved from N-hydroxylamines which did not react with nickel.

Amberlite XAD resins have also been used as reverse HPLC stationary phases for phenol analysis (230, 231, 237). Grieser and Pietrzyk (231) found that pH as well as buffer concentration affected the resolution of phenol, nitrophenols, alkylphenols and chlorophenols using an XAD column. XAD resin does not require a stationary liquid phase, therefore problems with column bleed are eliminated.

A number of detectors are available for HPLC. UV detectors are most frequently used at the common fixed wavelength of 254 nm (111, 150, 219, 228, 232); analyses at 280 nm (83, 110, 222), 215 nm (96), 230 nm (94) and 315 nm (222) have also been reported. Realini and Burce (110) detected 2-MCP, 2,4-DCP, 4-chloro-m-cresol, 2,4,6-TCP and PCP at 280 nm. This wavelength was superior to 254 nm for all chlorophenols tested except PCP. Hayes (219) compared the utility of the wavelengths 236 nm, 254 nm and 304 nm for the HPLC analysis of PCP. Maximum sensitivity was achieved at 254 nm; the other wavelengths were not suitable due to absorption by solvents and interfering material.

Electrochemical detectors provide increased sensitivity and improved specificity when compared to nonselective UV detectors.

Electrochemical detectors are not compatible with solvent programs due to instability. Their usefulness for the analysis of complex mixtures is, therefore, limited (39). Sternson and DeWitte analyzed ANI and its metabolites by HPLC using a UV detector at 254 nm and an electrochemical carbon paste detector (232, 233). Electrochemical detection was 5, 100 and 1000 times more sensitive than UV for 2-AP, ANI and 4-AP, respectively. Phenols are readily oxidized at carbon electrodes using potentials of +0.7 to +1.1 volts (V) (vs Ag/AgCl) (223). Sample contaminant interferences can be minimized by adjustment of the electrode potential. The comparatively high potential (+0.85 V) used by King et al. (238) to detect PCP minimized the response of many interfering materials. Mayer and Shoup (223) found electrochemical detection limits comparable to those achieved using ECD-GLC. Armentrout et al.(229) employed a new electrochemical detector with a carbon black polyethylene tubular anode to detect organic compounds at 1 ppb levels in water. The tubular anode could be used with any water-miscible organic solvent and gave the best signal-to-noise ratio of the several anodes tested. Phenolic compounds, present at less than 1 ppb, were successfully analyzed in wastewater samples. Dolan and Seiber (239) adapted the Coulson electrolytic conductivity detector for use with HPLC and applied the system to the analysis of organochlorine compounds including PCP.

Although sample cleanup procedures cannot be avoided in GLC and HPLC, the latter chromatographic technique usually does not

require derivatization. However, derivatives have been used in HPLC to improve detectability. Porcaro and Shubiak (240) prepared the p-methoxybenzoyl derivatives of hexachlorophene (2,2'-methylenebis[3,4,6-trichlorophenol]) to enhance UV absorption. At a wavelength of 254 nm the absorptivity of the derivative was sixty-four times greater than that of the parent compound.

d) Thin-Layer Chromatography

Although most analyses of phenols and anilines have utilized GLC and HPLC, a number of investigators have also reported the use of thin-layer chromatography (TLC) for semi-quantitative analysis with detection limits in the submicrogram range. Following chromatography on silica gel (97, 241-247), alumina (242, 243, 245) and a number of other stationary phases (248-253), spray reagents such as methyl orange, methyl red, methyl violet and 4-aminoantipyrine were used to detect as little as 1 µg PCP on the plates (246). Geike (247) reported a method for visualizing PCP on TLC plates based on the inhibition of enzyme activity. PCP, a strong inhibitor of numerous enzymes, could be detected at concentrations of 10 ng and 3 µg using bovine liver esterase and amylase, respectively. Dietz et al. (245) separated and identified one hundred and twenty-six phenolic compounds by TLC using six one dimensional thin layer systems and four spray reagents. The method was applied to the analysis of water samples containing phenols at ppb levels.

Derivatization of free hydroxyl groups is often used to improve TLC sensitivity and selectivity. Several workers (103, 241) have prepared dansyl derivatives of chlorophenols which could be detected by fluorescence measurements following TLC separation. Quantitative analysis was impossible using the method described by Van Langeveld (103) since a linear relationship between concentration and fluorescence intensity could not be demonstrated. The TLC detection limit for the PCP dansyl derivative was 4 ppm. Berbalk and Eichinger (254) converted phenols to colored compounds by reaction with p-(5-fluoro-2,4-dinitro-1-phenylazo)-N,N-dimethylaniline and separated the resulting derivatives by TLC.

Ting and Quick (255) developed a procedure designed to increase the specificity of analysis for PCP in a mixture of chloro-organics extracted from sawdust. PCP was converted to chloranil by warming briefly with concentrated nitric acid. Following TLC, PCP was detected as a blue spot by spraying with a citric acid solution of tetramethyl-p-diaminodiphenylmethane. Lower chlorophenols did not interfere with the detection of PCP but the procedure was not specific since TeCP reacted similarly to PCP.

In general, TLC is a simple method which provides non-specific, qualitative information on samples containing high concentrations of the compounds of interest. Mixtures containing several isomers or closely related compounds cannot be unequivocally identified, however. The major disadvantages

of TLC are comparatively low sensitivity, low specificity and poor quantitative results.

e) Spectrophotometry

Although colorimetric and UV ratio spectrophotometric methods are still used, their popularity continues to decline. Routine water quality monitoring for phenols is often based on nonspecific spectrophotometric tests such as the colorimetric 4-aminoantipyrine (4-AAP) method (256). Unfortunately, reagents such as 4-AAP do not react with equal sensitivity to all phenolics; para-substituted compounds are poorly detected (257). Colorimetric methods for quantitation of total phenol concentrations in natural waters are subject to a high degree of variability, thus limiting their value even as screening techniques. A number of oxidants have been reported to interfere with the 4-AAP and UV ratio spectrophotometric methods. Norwitz et al. (258) described a method for minimizing these interferences by the addition of sodium arsenite. Ramstad and Armentrout (125) analyzed total chlorophenols in brine by a modified 4-AAP reaction after selective XAD-2 column adsorption of the phenols from an aqueous sample. Phenols were eluted from the macroreticular resin with alkaline methanol. The 4-AAP test, performed in the presence of methanol, was applicable to solutions containing from 0.1 to over 2 mg/L total phenols. Anilines are usually considered as interfering compounds in the determination of phenolics (259) by the colorimetric 4-AAP

method. El-Dib et al. (260) investigated the use of 4-AAP for the colorimetric quantitation of anilines in natural waters. While the determination of some aromatic amines was possible at concentrations of 0.1 mg/L, 4-AAP was insensitive to chloro-, nitro- and p-substituted anilines. Koppe et al. (261) characterized the reaction of 4-AAP as well as three group-specific reagents, 4-nitroaniline, sulfanilic acid and 3-methyl-2-benzenethiazolinehydrazine, with one hundred and twenty-six phenolic compounds. Fountaine et al. (257, 262, 263) applied a UV ratio spectrophotometric method to the analysis of PCP in river water. The detection limit for PCP was 2 ppb with a recovery of 98%. While no interference was observed from 2-MCP, 3-MCP and 4-MCP, the presence of 2,4,6-TCP interfered with the specific analysis of PCP. Baker and Mayfield (112) monitored the microbial and nonbiological decomposition of chlorophenols in soil spectrophotometrically by the decrease in UV absorbance. In spite of instrumental advances and new chemical modifications, spectrophotometric methods remain limited in value since specific phenols cannot be identified and quantitated unequivocally at trace concentrations.

C. ANALYTICAL METHODS FOR MORPHINE AND ALKALOID DRUGS

1. Extraction

Extraction methods for phenolic alkaloids, such as morphine, are usually complex and require that the aqueous sample be maintained below pH 10 (the pKa of the phenolic group) using an appropriate buffer. Dahlstrom and Paalzow (264), for example, described a typical procedure for the recovery of morphine from biological material. The samples were extracted with toluene-butanol (9:1) at pH 8.9, re-extracted into 0.1N H_2SO_4 and, finally, back-extracted to a toluene-butanol phase at pH 8.9. Similar extraction sequences, using many different buffers and solvent combinations have been reported in the literature (265, 266).

Schill (267) first reported the use of bromothymol for ion-pair extraction of morphine. Recently, extractive alkylation methods have simplified both the extraction and derivatization of phenolic alkaloids. In the method reported by Cole et al. (268), the morphine phenoxylate was extracted into ethyl acetate as a tetrabutylammonium ion-pair in the presence of the alkylating agent pentafluorobenzyl bromide. The procedure allowed not only quantitative extraction but also simultaneous derivatization. Quantitation of plasma morphine concentrations as low as 5 ng/mL was reported.

2. Identification and Quantitation

Many methods are available for the separation, identification and quantitation of alkaloid drugs, including radioactive, spectrofluorometric and chromatographic techniques. GLC and HPLC analysis of these compounds will be considered in greater depth.

GLC analysis of underivatized morphine, ^{3}O-acetylmorphine, ^{6}O-acetylmorphine, diacetylmorphine and codeine have been reported (269-272). Although diacetylmorphine can be analyzed gas chromatographically at low concentrations without derivatization (271), the direct GLC detection of polar alkaloids containing free hydroxyl groups is limited to forensic or pharmaceutical samples containing high drug concentrations (269, 270). More sensitive and reliable GLC quantitative analysis of extracts of morphine and other polar alkaloid drugs has been achieved following alkylation (268), acylation (264, 273-281) and silylation with BSA (282-284), HMDS (285) or BSTFA (279, 283, 286).

Dahlstrom *et al.* (264, 273) prepared electron-capture sensitive derivatives of morphine using PFPA which allowed the GLC detection of 500 pg/mL in plasma and 100 pg/30 mg in brain tissue. The use of HFBA (274-276) and trifluoroacetic anhydride (TFAA) (277, 278) for derivatization of morphine, ^{3}O-acetylmorphine, ^{6}O-acetylmorphine and codeine has also been reported. While Yeh (279) observed that morphine derivatives prepared with TFAA were unstable, Wallace *et al.* (277) successfully used the reagent to quantitate morphine in serum and plasma. Ebbighausen *et al.* (276) found derivatization with TFAA

preferable to HFBA for the SIM-MS analysis of codeine, morphine, norcodeine and normorphine. Acetyl derivatives are less moisture sensitive than perfluoroacyl derivatives; morphine has been analyzed as its diacetyl derivative (279, 280). Acetylation methods, however, cannot be used for analysis of samples containing mixtures of diacetylmorphine, ^{3}O-acetylmorphine, ^{6}O-acetylmorphine and morphine itself. The use of propionic anhydride has rarely been reported in the literature. Anders and Mannering (281) prepared propionate and acetate esters of several alkaloid drugs by "on-column" acylation with the appropriate anhydride reagent. The flash-heat acylation method proved unsatisfactory for the derivatization of drugs containing two esterifiable groups; complex spectra with peaks corresponding to both monoacyl- and diacylmorphine were obtained.

HPLC has been applied successfully to the analysis of opiates using reverse-phase (287, 288) or paired-ion reverse-phase columns (289). UV detectors provide adequate sensitivity for the analysis of alkaloid drugs in forensic and pharmaceutical preparations without prior cleanup of the samples; optimal detection sensitivity is achieved at a wavelength of 280 nm (289). Frei *et al.* (288) derivatized the phenolic hydroxyl group of several alkaloid drugs with dansyl chloride (5-dimethylamino-1-naphthalene sulfonyl chloride) and detection was carried out simultaneously with a fluorescence detector and a UV detector set at 254 nm. The determination of therapeutic levels of morphine in small blood samples using HPLC with electrochemical detection was reported by White (287). The glassy carbon flow-through electrode was capable of detecting less than 1 ng of morphine extracted from blood samples.

D. SYNOPSIS

Prior to 1967, the procedures available for the analysis of phenols and anilines were primarily TLC or spectrophotometric methods. At that time, Bevenue and Beckman (166) concluded that these procedures were often tedious and failed to provide adequate sensitivity and specificity. Since then, the application of TLC and spectrophotometric methods has declined due to an increasing demand for procedures capable of unequivocal isolation, separation, identification and quantitation of multicomponent residues. The recoveries of trace organic compounds from sample materials have been improved by comparative investigations of solvent extraction efficiencies, the use of ion pair reagents and the development of efficient column adsorption techniques. Many new methods of derivatization for GLC have been described including procedures for the preparation of derivatives directly in aqueous solution. The use of capillary columns has improved the resolution of complex mixtures of organics and the introduction of new bonded stationary phases has lowered the limits of detection for underivatized phenols. Development of reliable electrochemical detectors, various modes of separation and on-column enrichment techniques have increased the utility of HPLC for trace analysis. The mass spectrometer has provided a means for unambiguous detection and identification of phenolic and anilino residues. New developments in analytical methodology continue to expand our understanding of the occurrence, metabolism, degradation, toxicity and environmental impact of trace organic compounds.

CHAPTER IV

MATERIALS AND METHODS

A. REAGENTS

1. Commercial Sources

Sodium bicarbonate, ether, acetone, ethyl acetate, methanol, toluene, methylene chloride, acetic anhydride, anhydrous sodium sulfate, cyclohexane, acetonitrile, chloroform, 2-propanol, sodium hydroxide, ammonium hydroxide, sulfuric acid, 1-NAP, 3-NP, 4-NP, PHE, o-CRE and m-CRE were obtained from Fisher Scientific Company (Fair Lawn, N.J., U.S.A.). Chloroacetic anhydride (CAA), dichloroacetic anhydride (DCAA), 2,4-DCP, p-CRE, 2-NP, 3- and 4-CA, ANI, 2,3,4,6-TeCP, 2-, 3- and 4-AP, 4-bromophenol (4-BrP), 4-chloro-3-methylphenol, 4-chloro-2-methylphenol, 3-chloro-4-methylaniline (3-Cl-4-MeA), 4,6-dibromo-o-cresol (4,6-DBC) and benzylamine (BA) were obtained from Eastman-Kodak Company (Rochester, N.Y., U.S.A.). Matheson, Coleman and Bell (Norwood, OH., U.S.A.) was the source of 2-MCP, 2,4-DCP, 6-chlorothymol and catechol while naphthalene was obtained from Anachemia Company (Montreal, Canada). 4-Bromoaniline (4-BrA), 4-MCP, 4-chloro-3,5-dimethylphenol, diamorphine hydrochloride, codeine sulfate and morphine sulfate were obtained from British Drug Houses Ltd. (Poole, England). Aldrich Chemical Company (Milwaukee, Wis., U.S.A.) was the source of PFBC, eugenol, isoeugenol and 2,6-DCP while benzo(a)pyrene was obtained from RFR Corp. (Hope, R.I., U.S.A.). Mallinkrodt Inc. (St. Louis, Mo., U.S.A.) provided Amberlite XAD-2 resin (20-50 mesh). TFAA, PFPA and HFBA were obtained from Sigma Chemical Company (St. Louis, Mo., U.S.A.).

Methylene chloride was distilled before use. Sodium sulfate and glass wool were heated for 3 hr in a muffle furnace at 400 °C and stored in capped (aluminum foil-lined) amber bottles.

2. U.S. Environmental Protection Agency Reference Standards

The phenols listed in Table IV were obtained as high-purity analytical grade reference standards from the U.S. Environmental Protection Agency, Quality Assurance Section, Analytical Chemistry Branch, HERL, ETD (MD-69), Research Triangle Park, N.C. 27711 U.S.A. The compounds were ordered from the stock index catalogue entitled "Analytical Reference Standards and Supplementary Data for Pesticides and other Selected Organic Compounds".

3. Distilled Water

Deionized, glass-distilled water was prepared using a Corning AG-11 distillation unit (Corning Glassworks, Corning, N.Y., U.S.A.).

B. APPARATUS

1. Gas-Liquid Chromatography

Capillary GLC analysis was performed using a Hewlett Packard Model 5830A gas chromatograph equipped with a 15 mCi ^{63}Ni source

Table IV. Analytical Grade Reference Standards Obtained from the U.S. Environmental Protection Agency.[1]

Catalogue Code Number	Compound (index name)	Catalogue Code Number	Compound (index name)
P403	2,3-dimethylphenol	P645	o-hydroxyacetophenone
P404	2,4-dimethylphenol	P646	m-hydroxyacetophenone
P405	2,5-dimethylphenol	P647	p-hydroxyacetophenone
P505	2-ethylphenol	P974	2,4,6-trichlorophenol
P506	3-ethylphenol	6890	2,4,5-trichlorophenol
P507	4-ethylphenol	P920	2,3,4,5-tetrachlorophenol
P565	o-methoxyphenol	5260	pentachlorophenol
P704	2-isopropylphenol	0822	4-bromo-2,5-dichlorophenol
P705	3-isopropylphenol	4116	3-trifluoromethyl-4-nitrophenol sodium salt
P706	4-isopropylphenol		

1. 100 mg of each standard was supplied in glass vials with teflon-lined screw caps.

linear ECD and coupled to a Model 18850A integrator. For ECD-GLC analysis with packed columns, a Hewlett Packard Model 5730A chromatograph equipped with a 15 mCi ^{63}Ni linear ECD and Model 3380A integrator was used. Flame ionization detection (FID) GLC analysis with packed columns was conducted using either a Perkin-Elmer 990 or a Hewlett Packard Model 5702A chromatograph coupled to a Hewlett Packard Model 3380A integrator. All gas chromatographs were operated at injector and detector temperatures (^{o}C) of 250° and 275°, respectively.

2. Mass Spectrometry

a) Electron Impact-Mass Spectrometry

Electron impact mass spectra were recorded using a combined Hewlett Packard Model 5710A gas chromatograph/Model 5981A mass spectrometer/Model 5934A data system. The mass spectrometer was used in both the total ion mode and selected ion monitoring mode. MS scan conditions: Scan speed, 100 AMU/sec; bandwidth, 430 Hz; electron energy, 10-70 eV; dwell time, 200 msec; ion source temperature, 180°C. Separator temperature was the same as the gas chromatograph oven temperature.

b) Chemical Ionization-Mass Spectrometry

Chemical ionization mass spectra were obtained using a Hewlett Packard Model 5985A combined gas chromatograph/mass spectrometer with dual EI/CI sources and data system. MS scan

conditions: electron energy, 150 eV; CI methane carrier gas, 4 mL/min; ion source pressure, 0.6 Torr.

3. Gas-Liquid Chromatographic Columns

Capillary GLC was performed using a 10 metre (m) SP-2100 (0.8 mm o.d., 0.25 mm i.d.), WCOT capillary column (J&W Scientific, Inc., Orangevale, CA, U.S.A.). Helium at 7 psi was the carrier gas and 10% methane in argon at a flow rate of 36 mL/min was used as the makeup gas of the detector.

The packed columns listed in Table V were used for GLC and GLC-MS at a helium carrier gas flow rate of 60 mL/min. The glass columns and glass wool plugs were deactivated by silanization using a published procedure (290). SP-1240 DA is a packing specially deactivated with phosphoric acid. After continued use, the H_3PO_4 volatilized from the packing; rejuvenation was accomplished by the injection of 10-20 µL of 5% H_3PO_4 in acetone at 140°C.

4. Glassware

a) Kuderna-Danish Evaporator

Large volumes of organic extracts were concentrated by distillation using a Kuderna-Danish evaporator (291) (Figure 4) obtained from Ace Glass Co. (Vineland, N.J., U.S.A.). The

Table V. Packed Columns Used for Gas-liquid Chromatography[1,2,3]

Liquid Phase	Support	Mesh	Length (m)
5% OV-101	Chromosorb W	(80-100)	1.26
3% OV-17	Chromosorb W	(80-100)	1.68
1% SP-1240 DA	Supelcoport	(80-100)	1.26
0.2% Carbowax 20M	Glassbeads	-	0.84
0.1% SP-1000	Carbopack C	-	0.84
3% OV-17	Chromosorb W	(100/120)	0.84
3.8% OV-17	Chromosorb W	(100/120)	1.68

1. Operating temperatures for each column are described in the text.

2. All columns were 4 mm i.d. glass tubing.

3. For gas chromatographic analysis FID was used with packed columns, unless otherwise stated.

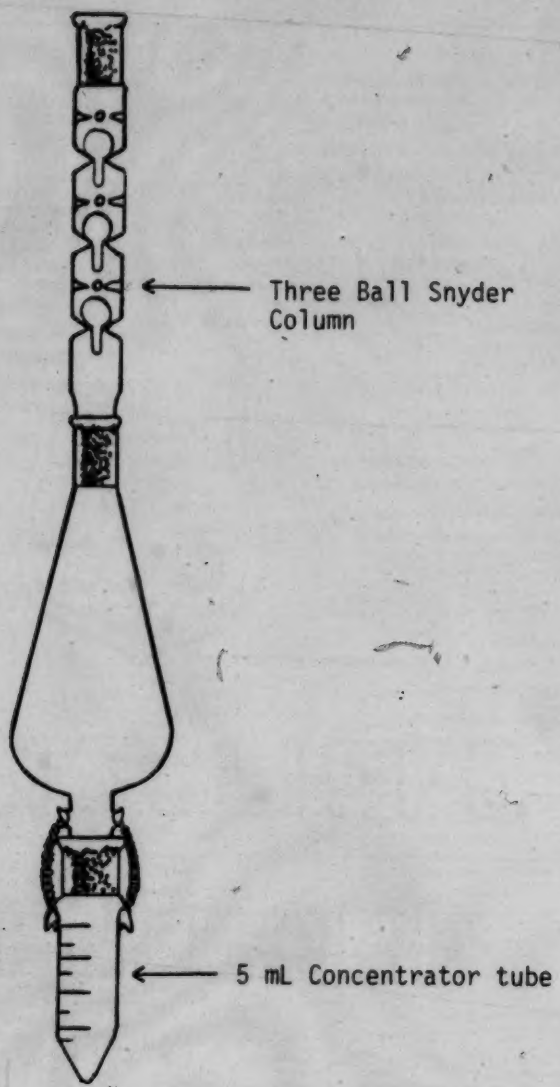


Figure 4. Kuderna-Danish evaporative concentrator (Ref. 25, 291).

solvent volume was reduced by placing the evaporator in a steam bath according to a published procedure (25).

b) Cleaning of Laboratory Glassware

Bevenue et al. (132) compared the effectiveness of the four procedures listed in Table VI for the cleaning of laboratory glassware used in trace analysis. Method I failed to completely remove glassware contaminants while methods II, III and IV equally efficiently removed all organic material. Organic solvents alone do not remove firmly adsorbed organic contaminants from glass surfaces. Treatments with oxidizing reagents, concentrated mineral acids or heat are considered essential for the preparation of contaminant-free glassware. In this study, glassware was washed with tap water and Alconox and was then sequentially rinsed with distilled water, ethanol and acetone. After air-drying, the glassware was heated in a muffle furnace (Model F1730 Serial #15227, Thermo Electric Mfg. Co., Dubuque, Iowa), for 3 hr at 400°C.

C. STANDARD SOLUTIONS

Individual standard stock solutions (1.0 mM) of all of the simple phenols and anilines listed under Reagents in Section "A" were prepared in 95% ethanol and stored in glass-stoppered bottles at 4°C. The standard stock solutions were diluted 10-100 times with distilled water to prepare "working standard" solutions. The working solutions

Table VI. Glassware Cleaning Procedures (see Ref. 132)

Method			
I	II	III	IV
Ethanol [1]	Dichromate-H_2SO_4 [2]	Acetone	Dichromate-H_2SO_4 [2]
Acetone	Tap water		Tap water
Hexane	Distilled water		Distilled water
	Acetone		Acetone
Air-dry	Air-dry	Air-dry	Air-dry
		Heat [3]	Heat [3]

1. Glassware rinsed three times with each solvent in order of the listed sequence.

2. Glass was soaked for 16 hr in a solution of sodium dichromate-concentrated sulfuric acid.

3. Glass was heated in an air oven for 16 hr at 200°.

were prepared at concentrations which allowed the addition of convenient and easily measured (0.1-1.0 mL) volumes of standards to distilled water or biological, environmental and forensic sample materials. The working solutions were used to prepare calibration graphs for quantitative analysis as well as to determine the minimum detectable concentration level of a developed procedure.

A stock solution of benzo(a)pyrene (1 mg/mL) was prepared in benzene.

D. XAD-2 RESINS

1. Preparation

Amberlite XAD-2 resin (20-50 mesh), supplied in a saturated sodium chloride solution, was washed before use. The resin was rinsed four times by suspending in distilled water and decanting. This was followed by two washes each with four times the resin volume of acetone, two washes with methanol and finally three resin volumes of distilled water. The resin was kept refrigerated in distilled water prior to use. The purified XAD resin, as a distilled water slurry, was poured into a 25 mL buret (1 cm i.d.) containing a silanized glass wool plug near the stopcock. The slurry was allowed to settle until a resin bed approximately 6 cm high was obtained (1.5-2.0 g dry resin). A second silanized glass wool plug was

inserted above the resin bed and the completed column was washed with three 20 mL portions of distilled water.

2. Use

A 250 mL water sample, containing 0.5 mg each of 2-NP, PHE, m-CRE and 2,4-DCP was passed through a prepared XAD-2 resin bed at a flow rate of 5 mL/min. The column was then eluted with 10 mL of solvent (acetone, methylene chloride or ether). The first 5 mL of solvent was allowed to run through the resin slowly until only a small amount remained above the upper glass wool plug. The column was capped with an aluminum foil-covered stopper and allowed to stand undisturbed for 10 min. The second 5 mL aliquot was then passed through the resin bed slowly and the combined column effluents were reduced to a 100 µL volume using a gentle stream of nitrogen gas.

E. PREPARATION OF SAMPLES AND STANDARDS FOR DERIVATIZATION

1. Urine Samples

a) Analysis of Anilines and Aminophenols

Five mL volumes of urine were added to 15 mL teflon-lined screw capped test tubes. Following the addition of 0.2 mL concentrated H_2SO_4, the tubes were tightly sealed and placed in a boiling water bath for 1 hr. The hydrolyzed samples were

cooled and 100 nmoles of internal standard BA was added to each sample. The samples were then extracted twice with 2 mL volumes of methylene chloride. The methylene chloride extracts were discarded and the pH of each urine sample was adjusted to near pH 7.0 by the addition of 1.2 mL 10N NaOH. These samples were then derivatized with acetic anhydride and TFAA.

In order to determine the efficiency and sensitivity of the procedure developed for the analysis of anilines in human urine, known concentrations of ANI, 3-CA, 4-CA, 3-Cl-4-MeA and 4-BrA were added to urine samples naturally containing no detectable aniline residues. Following the addition of 1-100 nmoles of each aniline to 5 mL aliquots of urine, the samples were hydrolyzed and further processed as described above.

Two calibration graphs were constructed for each aniline and aminophenol using the internal standard addition method; one graph for the packed 3% OV-17 column with flame ionization detection and the second for the SP-2100 capillary column with electron capture detection. The minimal detectable concentration of each compound for the above GLC systems was also determined. For FID calibration, 50 nmoles of BA and 1-500 nmoles of 2-, 3- and 4-AP, ANI, 3-CA, 4-CA, 3-Cl-4-MeA and 4-BrA were added to 100 mL distilled water samples. For ECD calibration, 5 nmoles of BA and 0.05-20 nmoles of each aniline listed above were added to 100 mL distilled water samples. Similarly for ECD calibration of APs, 2.5 nmoles of BA and from 0.10-500 nmoles of 2-, 3- and 4-AP and ANI were added to 100 mL distilled water samples.

b) Analysis of Chlorophenols by Selected Ion Monitoring-Mass Spectrometry

Urine samples (5 mL) were hydrolyzed and cooled as described in a). Each sample, following the addition of 0.2 nmoles of 4,6-DBC as internal standard, was basified to pH 12 with NaOH pellets and extracted twice with 2 mL aliquots of methylene chloride to remove basic and neutral organics. The organic layers were discarded and the pH of the partially purified urine sample was adjusted to near pH 7.0 with concentrated H_2SO_4 prior to derivatization with acetic or propionic anhydride.

2. Environmental Water Samples

Water samples were collected in pre-cleaned glass bottles with aluminum foil-lined caps and stored refrigerated at 4°C. They were usually analyzed within 1-12 hr of collection. Details concerning sample collection and handling in each study are described individually below. In each case, following the addition of internal standard, the sample was basified to pH 12 with NaOH pellets and extracted with three 25 mL aliquots of methylene chloride to remove basic and neutral interfering compounds. The pH of the aqueous phase was then adjusted to 7.0 with concentrated H_2SO_4 prior to derivatization.

a) Study of Nitrophenols in the Athabasca River

Athabasca River water samples were collected at three different sites near Fort McMurray, Alberta. To identify naturally occurring phenolics, 750 mL samples of Athabasca River water, with 0.1 μmoles of 1-NAP internal standard added, were acetylated. To determine the stability of phenols in Athabasca River water, 400 mL samples were spiked with 1 μmole/L each of 2-NP, 4-NP and m-CRE. The samples, placed in 1-L Erlenmeyer flasks, were stoppered with sponge and shaken at 20°C using a New Brunswick Model G2 Shaker (New Brunswick Scientific Co. Inc., Edison, N.J.). Aliquots (50 mL) were removed at intervals over a two-week period and frozen until analyzed. For quantitative analysis, 0.05 μmoles of 1-NAP internal standard was added to each aliquot prior to base neutral methylene chloride extraction and derivatization with acetic anhydride. Calibration graphs were constructed by the addition of 0.02-0.2 μmoles each of 2-NP and 4-NP and 0.1 μmoles of internal standard 1-NAP to 250 mL aliquots of distilled water.

b) Identification and Quantitation of Phenols in North Saskatchewan River Water and Edmonton Municipal Snow Dumps

Sampling of the North Saskatchewan River and Edmonton municipal snow dump sites was conducted, as described below, by the City of Edmonton Department of Engineering. Grab samples of the North Saskatchewan River were collected up- and downstream from a municipal snow dump site on three consecutive days (March 4, 5, 6, 1981). Holes were drilled through the river ice and samples were taken approximately 8" below the ice surface level.

Each sampling consisted of two 1-gallon amber glass bottles filled with river water. One bottle was preserved immediately by the addition of 1.0 g $CuSO_4 \cdot 5H_2O$/L and H_3PO_4 to pH 4.0; no preservatives were added to the second sample. Two composite samples, one preserved and one unpreserved, were also prepared by pooling equal volumes of river water collected on the three sampling days.

Snow samples, labelled SDX-81-1, SDX-81-2 and SDX-81-3, were collected at three municipal snow dump sites. Holes were drilled into the snow dump using a 4" diameter ice auger. The top 12" of the dump sample was discarded due to melting on previous days. Holes varied in depth from 7' to 16' depending on the height of the snow above ground level. Each site was cored at several random locations. The cores from each site were combined, thawed together and pooled. The melted snow, containing mud, sand and solid debris, was centrifuged and the clear supernatant was analyzed. To each 1 L sample, 50 nmoles of 4,6-DBC internal standard was added prior to base neutral extraction and acetylation. Calibration graphs were prepared by adding 1-200 nmoles each of PHE, o-CRE and p-CRE and 50 nmoles of 4,6-DBC internal standard to 1 L samples of distilled water.

c) Analysis of Simple Phenols in Raw and Treated North Saskatchewan River Water

Raw and treated North Saskatchewan River water samples were obtained from the Rossdale Water Treatment Plant (Edmonton, Alberta) on January 19, March 2 and June 1, 1981. These water

samples were expected to contain very low phenol concentrations, therefore, 3-L sample volumes were processed. Each 3-L sample, to which 50 nmoles of internal standard 4,6-DBC had been added, was divided into 1-L aliquots. The aliquots were individually extracted with methylene chloride to remove base-neutral interfering compounds and acetylated. Following derivatization, each 1-L volume was extracted individually; the extracts were then recombined in order that the original 3-L volume of water could be gas chromatographically analyzed for phenolic content as a single sample. Calibration graphs were prepared by adding 1-10 nmoles each of PHE, o-CRE and p-CRE and 50 nmoles of 4,6-DBC internal standard to 1-L samples of distilled water.

d) Analysis of Phenols in Industrial Waters

As shown in Figure 5, the Syncrude Canada Ltd. oil sands extraction plant is located approximately 50 km north of Fort McMurray and 420 km north of Edmonton, Alberta. The water samples listed in Table VII were provided by Dr. M.D. McKinnon (Environmental Affairs Department, Syncrude Canada Ltd., Edmonton, Alberta). Water was collected from the sampling sites in and surrounding the tailings pond as shown in Figure 6. The samples, divided into four basic groups according to their source, are described in detail below.

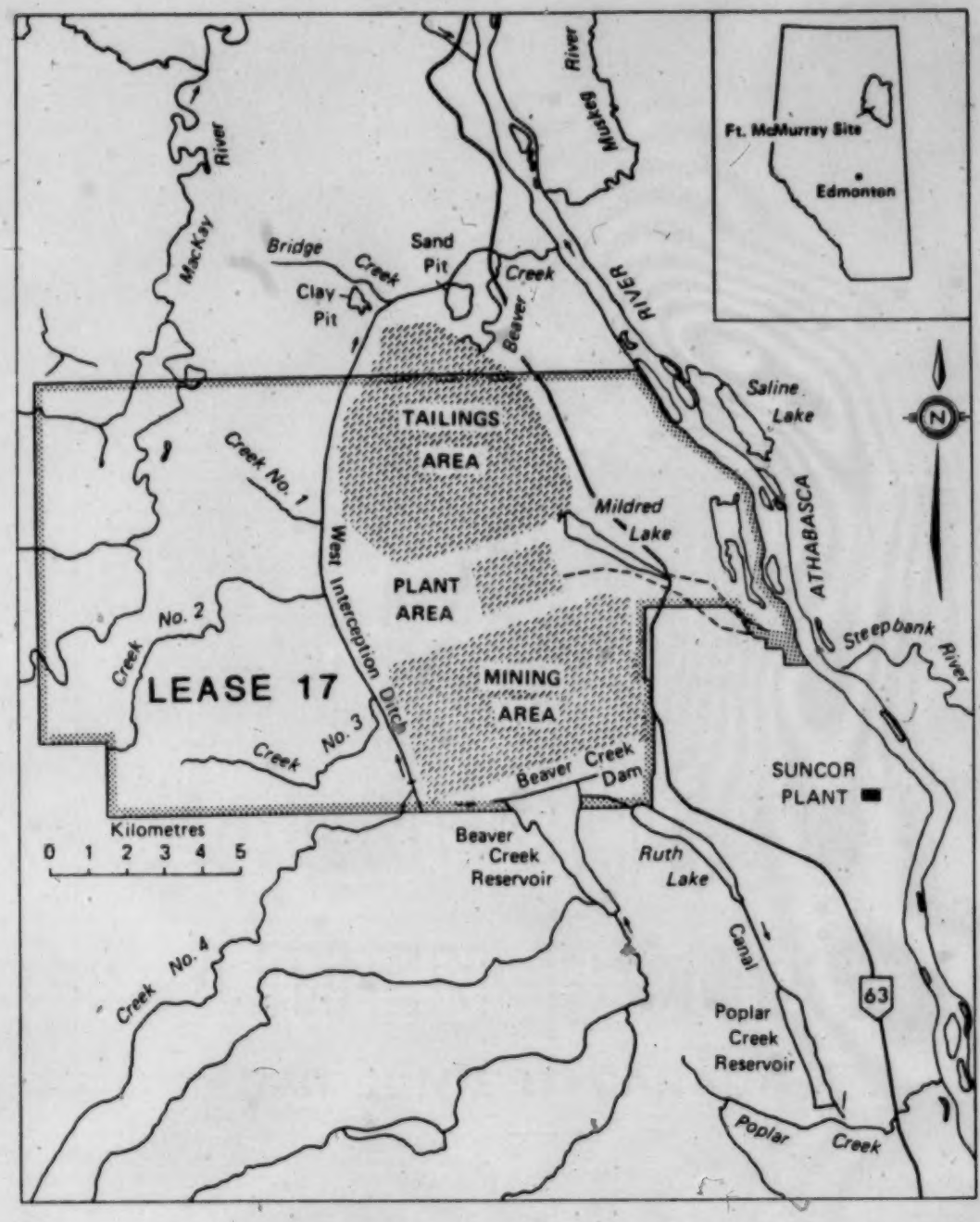


Figure 5. Syncrude Canada Ltd. oil sands extraction plant on Lease No. 17, north of Fort McMurray, Alberta.

Table VII. Process-Affected Waters and Natural Waters Collected in and Surrounding the Syncrude Canada Ltd. Tailings Pond

Sampling Date							
21.4.81[1]		27.4.81[2]		11.6.81[1]		8.7.81[1]	
Sample Number	Source	Sample Number	Source	Sample Number	Source	Sample Number	Source
1	Dyke drainage	8	Tailings Pond (depths) 2 m	16	Sand pit	23	Piezometer OW27R
2	Dyke drainage	9	6 m	17	Catchment basin	24	Piezometer OW12R
3	Dyke drainage	10	10 m	18	Piezometer OW12R	25	Piezometer OW3R
4	Dyke drainage	11	15 m	19	Beaver Creek	26	Piezometer T-14
5	Collector ditch	12	2 m	20	Piezometer OW3R	27	Piezometer OW18R
6	Catchment basin	13	6 m	21	Piezometer T-2	28	Tar sand extraction water
7	Sand pit	14	10 m	22	Mildred Lake	29	Natural drainage over oil sand

1. 1-L samples were analyzed.
2. 100 mL samples were analyzed.

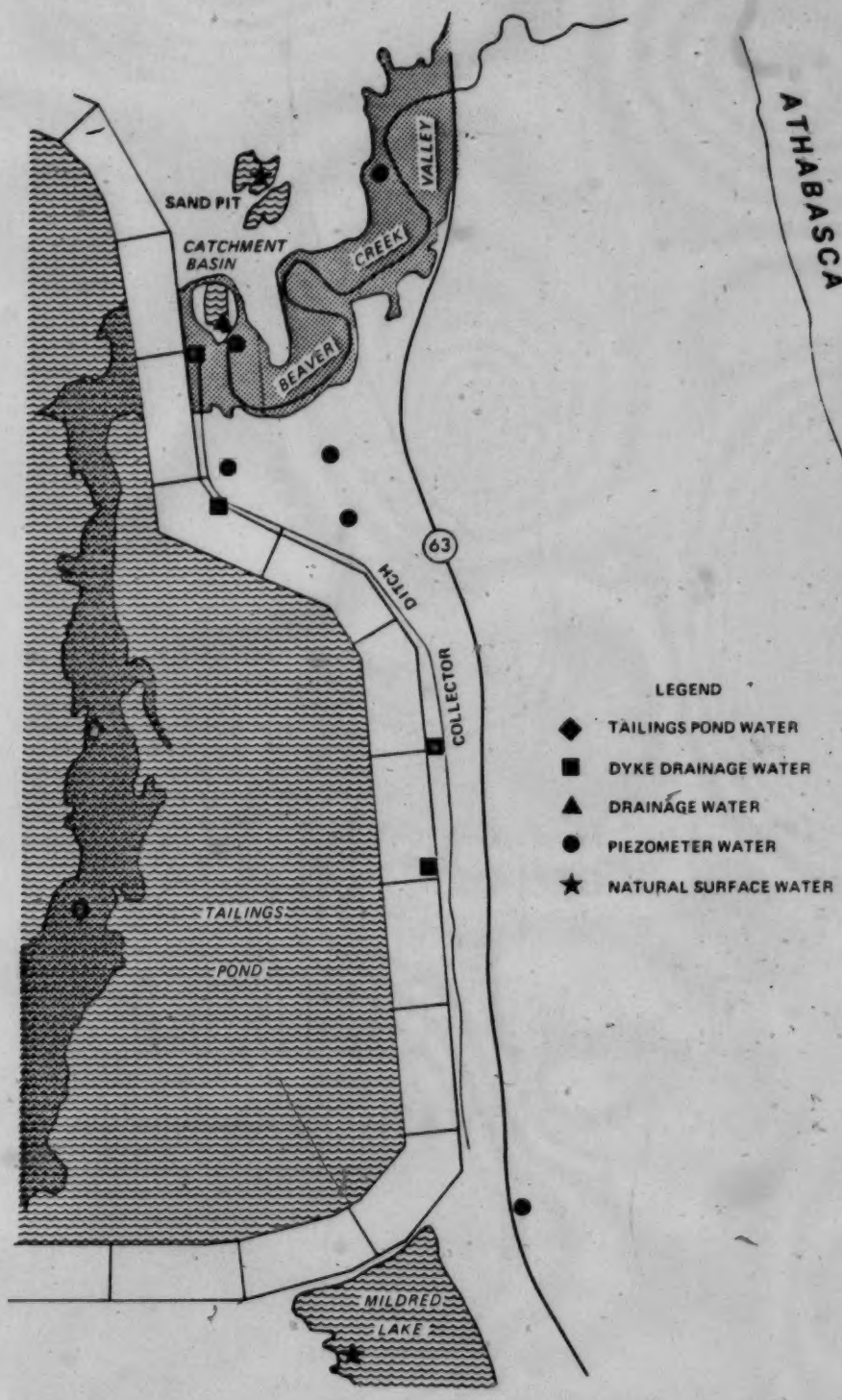


Figure 6. Location of sampling sites in and surrounding the Syncrude Canada Ltd. tailings pond.

1. Waters from or affected by the tailings pond.

a) Tailings pond water. Water used in the production of synthetic crude oil from bitumen is stored in a "tailings pond". The water is surrounded by sand dykes designed to isolate the process-affected waters containing sand, clay, bitumen, process reagents and trace components of environmental concern. Water samples were taken from 2, 6, 10 and 15 m depths of the pond to provide a profile of the water quality at various levels.

b) Dyke drainage water. Filter systems, consisting of a gradation of course gravel and fine sand, are incorporated in the tailings pond dykes. These filters allow the removal of water in the dyke structure. The water from these filters drains into a collection ditch.

c) Collector ditch water. Any water running out of or off the dykes will be caught by a collector ditch which runs along the outside of the dyke. The composition of the ditch water is mainly drainage from the dyke filters with some surface runoff.

d) Catchment basin water. The water from the collector ditch runs into a catchment basin (i.e. seepage control pond) from which it is pumped back into the tailings pond.

e) Oil sand extraction water. A sample of oil sand was extracted on a laboratory bench scale using hot water. The extract water was prepared for comparison to tailings pond water (produced by extraction of oil sand with hot water containing NaOH).

2. Beaver Creek Water. The catchment basin is dammed to prevent water from entering Beaver Creek, a natural stream. Beaver Creek is in close proximity to process-affected waters, however, and is susceptible to seepage from the tailings pond.

3. Natural Surface Water. "Natural" waters are defined as water bodies which are formed by natural drainage or precipitation. These sources provided background values of natural waters and an indication of the variability of waters in the area. The "Sand Pit" is a large standing water body under no direct influence from process waters at the time of this study. Mildred Lake, fed by the Athabasca River, is the fresh water source for the Syncrude Canada Ltd. extraction plant. Lastly, one sample of natural drainage water flowing over an oil sand deposit was collected. This water sample provided information concerning the effect of the unprocessed oil sand on surface water quality.

4. Piezometer water. Piezometers, installed on the dyke and in areas outside of the collector ditch, monitor groundwater pressure. Piezometers consist of 37 mm diameter polyvinyl chloride pipe; the pipe is slotted at the bottom to allow groundwater to flow in and accumulate. These water samples provide an indication of groundwater quality in an area close to the dyke system.

Water samples (1-3 L) were collected using a vacuum pump system. The samples were transferred directly to pre-cleaned bottles, with care being taken to prevent contamination of the water by the pumping mechanism. Tailings pond water samples were centrifuged to remove suspended solids prior to analysis. Internal standard 4-BrP (0.1 μmoles) was added to each sample listed in Table VII prior to base-neutral extraction and acetylation. As shown in Table VII, several samples were collected in duplicate on different sampling dates in order to check the reproducibility of both the sampling and analytical procedures.

Calibration graphs for PHE, o-CRE and p-CRE were constructed by adding 0.001-0.40 μmoles of each phenol and 0.10 μmoles of internal standard 4-BrP to 1 L distilled water samples.

3. Forensic Samples

Eight street dosage drug preparations were examined for the presence of morphine and heroin. Triplicate 5 mg samples of each oily brown residue were dissolved in 1.0 mL aliquots of distilled water containing approximately 0.2 g $NaHCO_3$. Similarly, for quantitative analysis of morphine, standards were prepared by dissolving 0.1-1.0 mg of authentic morphine in 1 mL aliquots of distilled water containing $NaHCO_3$. To all aqueous samples and standards, 0.2 mg of benzo(a)pyrene internal standard was added. For the determination of heroin, one dissolved replicate sample was

extracted twice with 1 mL volumes of chloroform: 2-propanol (9:1). The organic solvent extract was concentrated to 100 µL using a gentle stream of nitrogen prior to GLC analysis. The remaining two replicate samples and the standard solutions were acylated with acetic or propionic anhydride (described in section F) for the GLC determination of morphine as its acetate or propionate ester.

F. BASIC DERIVATIZATION PROCEDURES

1. Aqueous Acylation

a) In situ Method

Simple phenols and anilines, as well as morphine, were acylated directly in aqueous solution with acetic anhydride or propionic anhydride. The acylation-extraction procedure was adapted to the analysis of 100-1000 mL water samples, 5 mL urine samples and small aqueous sample volumes containing morphine. Unless otherwise stated, ratios of 1 mL water sample : 20 mg $NaHCO_3$: 1 µL acyl anhydride were used.

Water samples (100-1000 mL) were derivatized directly in separatory funnels of appropriate size. Derivatives were prepared by the addition of the excess $NaHCO_3$ followed by the addition of acetic anhydride or propionic anhydride to the aqueous sample. After the evolution of carbon dioxide ceased, the acyl derivatives were extracted by shaking the solution

three times for 2 min each with 25 mL volumes of methylene chloride. After combining the extracts, traces of water were removed by the addition of anhydrous Na_2SO_4 (1-2 g). The total extract was then concentrated to a volume of approximately 5 mL using a Kuderna-Danish evaporator (25). The solvent was further reduced to 100 µL under a gentle stream of nitrogen. A 1 µL sample was analyzed gas chromatographically. Evaporation of sample extracts to dryness was avoided since erratic results were obtained due to the loss of volatile acyl derivatives.

In a typical complete procedure, a 1 L water sample was transferred to a 1 L separatory funnel and internal standard was added. The pH of the solution was adjusted to greater than pH 12 by the addition of NaOH pellets (3-5 pellets) and the base-neutral interfering compounds were extracted three times with 25 mL volumes of methylene chloride. The methylene chloride was discarded and the pH of the aqueous phase was adjusted to near pH 7 by the addition of concentrated H_2SO_4. For acetylation, 20 g $NaHCO_3$ and 1.0 mL of acetic anhydride were added. After vigorous shaking with frequent venting of the separatory funnel to permit the release of evolved carbon dioxide, the sample was allowed to stand for 2-5 min to allow completion of the reaction. The aqueous phase was then extracted three times with 25 mL volumes of methylene chloride. The combined extracts were dried with Na_2SO_4 and the solvent volume was reduced to 100 µL using a Kuderna-Danish evaporator followed by a gentle stream of nitrogen, as previously described.

Urine samples (5 mL) were acylated with acetic or propionic anhydride in 15 mL teflon-lined screw capped test tubes in a similar manner. The acyl derivatives were extracted by shaking the urine samples twice for 2 min with 2 mL of methylene chloride. After combining the extracts, traces of water were removed by passing the methylene chloride through a Pasteur pipet containing a glass wool plug and approximately 1 g of anhydrous Na_2SO_4. The Na_2SO_4 column was rinsed with a further 0.5 mL of methylene chloride and the dried extract was concentrated to 100 µL using a gentle stream of nitrogen.

As described in Section E, forensic samples and morphine standards were dissolved in distilled water containing $NaHCO_3$; the solutions were acylated by the addition of 0.1 mL of propionic or acetic anhydride. When the acylation reaction was complete (2-5 min), the solutions were extracted twice with 1.0 mL aliquots of chloroform : 2-propanol (9:1). The organic solvent extracts were concentrated to 100 µL using a gentle stream of nitrogen.

b) Phase Transfer Method

To a 2 mL aqueous solution containing 0.01-200 µg of morphine, 200 mg of Na_2CO_3 and 4 mL of ethyl acetate : acetonitrile (9:1) containing 2 µL of PFBC were added. The phase transfer reaction was allowed to proceed to completion (5 min) with constant vortex mixing. The organic layer was removed and dried with Na_2SO_4 as described for urine samples in method a).

The extract was then evaporated to dryness under a gentle stream of nitrogen and the residue was redissolved in 100 µL of toluene. To remove traces of unreacted PFBC, the toluene layer was mixed by vortex for 15 sec with 200 µL of 10 N NH_4OH solution. The toluene layer was then analyzed by ECD-GLC using a 3% OV-17 column (0.84 m) at 280°C, isothermal.

2. Perfluoroacylation

N-Acylated BA (internal standard), ANI, 3-CA, 4-CA, 4-BrA and 3-Cl-4-MeA, N,O-diacylated 2-, 3- and 4-AP isomers and ^{3}O-acylated morphine were further reacted with TFAA, HFBA or PFPA to produce derivatives with enhanced electron capture detection sensitivity. To 100 µL of the extract containing the acylated compounds, obtained as described in the *in situ* acylation procedure, 50 µL of the appropriate perfluoroanhydride was added. The mixture was capped, vigorously shaken for 15 sec, and then allowed to react at room temperature for 15 min. One mL of cyclohexane was added and the sample volume was concentrated to 100 µL under a gentle stream of nitrogen prior to GLC analysis.

CHAPTER V

RESULTS AND DISCUSSION

A. METHOD DEVELOPMENT - ANALYSIS OF STANDARD MIXTURES

1. Recovery of Phenols from Aqueous Solution

Methods for the determination of trace concentrations of phenols in aqueous samples generally include an extraction and/or concentration step prior to GLC analysis. Direct solvent extraction and XAD resin adsorption are two commonly used methods. In this study, the extraction efficiencies of these two methods were compared to that achieved by liquid-liquid extraction of phenols as acetate derivatives prepared directly in aqueous solution. As shown in Table VIII, the recoveries of PHE and m-CRE from 250 mL of water by liquid-liquid extraction with 15 mL of methylene chloride were less than 70%. Recoveries of the phenolics by adsorption on XAD resins and subsequent elution with acetone, ether or methylene chloride were very similar. None of these methods recovered even 50% of PHE in the mg/L concentration range. Recoveries of all the phenols listed in Table VIII would be expected to worsen substantially at lower concentration levels. Van Rossum and Webb (292), for example, reported recoveries of 14-46% and 33-69% for PHE and p-CRE, respectively, at the 50 µg/L level using XAD resin adsorption. One objective of the present study was to keep the volume of extracting solvent as low as possible and when this was done (Table IX), efficiency of recovery was found, as expected, to be directly related to the concentration of the phenol in aqueous solution. When, for example, 1.0 mL of water which contained 94 µg of PHE was extracted

Table VIII. Recovery of Phenols (0.5 mg/250 mL) from Water by Liquid-liquid Extraction and Resin Adsorption

	RECOVERY (%)			
		XAD-2 Resin Adsorption		
Compound	Liquid-liquid Extraction Methylene Chloride	Acetone	Ether	Methylene Chloride
2-nitrophenol	93.2	98.0	101.0	94.0
phenol	43.4	45.4	43.7	41.[illegible]
m-cresol	66.9	75.8	68.0	70.0
2,4-dichlorophenol	80.0	84.0	78.0	83.0

Table IX. Effect of Concentration and Solvent:Water Ratio on the Extraction Efficiency of Phenol and Phenyl Acetate

Concentration (mM)	Water : CH_2Cl_2 Ratio	Concentration (µg/mL)		Recovery (%)	
		Phenol	Phenyl Acetate	Phenol[1]	Phenyl Acetate
1.0	1:1	94	136	100	100
0.1	10:1	9.4	13.6	50.8	93.6
0.05	20:1	4.7	6.8	42.2	106.7
0.002	50:1	0.188	0.272	28.3	100.0

1. A calibration curve was constructed to determine phenol recoveries using naphthalene as the internal standard.

with 1.0 mL of methylene chloride, virtually all of the phenol entered the organic phase. However, when 10 mL of water containing 9.4 μg/mL PHE was similarly extracted, only 51% of the phenol was detected in the organic solution. In contrast, the recovery of PHE as phenyl acetate, following aqueous acetylation, remained quantitative at all concentrations and water : solvent ratios examined.

The aqueous acetylation method was applied to the analysis of four synthetic mixtures containing 0.02-0.06 μmoles of each phenol and 0.05 μmoles of 1-NAP internal standard. The amount of phenols added and found by extraction are shown in Table X. The recoveries ranged from 90-104% and demonstrated that the method could accurately quantitate low concentrations of phenols in aqueous solution.

2. Derivatization and GLC Analysis of Simple Phenols

While many of the existing methods for phenol analysis are capable of detecting ppb concentrations, these procedures have three major disadvantages. They are time consuming; they require that the phenols be removed from the aqueous solutions by extraction with an organic solvent; and although many procedures are suited to the quantitation of trace amounts of phenols in small volumes of urine, they are not readily adaptable to the analysis of microgram quantities of phenolic compounds in large volumes (up to 1 L) of aqueous solution. Extraction of aqueous solution by organic solvents or adsorption and elution from macroreticular resins (Tables VIII and

Table X. Recovery of Phenols as Acetates from 250 mL Aqueous Solution

	Phenols			
	Phenol	o-Cresol	m-Cresol	2,4-Dichlorophenol
Added (μmoles)	0.035	0.030	0.060	0.035
Found (μmoles)	0.035	0.029	0.056	0.032
Recovery (%)	100	97	93	92
Added (μmoles)	0.025	0.020	0.040	0.0250
Found (μmoles)	0.026	0.019	0.041	0.0255
Recovery (%)	104	95	102	102
Added (μmoles)	0.060	0.050	0.020	0.055
Found (μmoles)	0.058	0.046	0.020	0.055
Recovery (%)	97	92	100	100
Added (μmoles)	0.020	0.035	0.035	0.0200
Found (μmoles)	0.020	0.032	0.034	0.0205
Recovery (%)	100	90	97	102

IX) resulted in a low recovery of phenolic compounds. In contrast, trace amounts of some methyl- and chlorophenols (Table X) could be directly acetylated in water and the resulting esters quantitatively extracted and analyzed by GLC. In this study, the aqueous acylation method was applied to the GLC analysis of the forty-one phenols listed in Table XI. Nine of the eleven phenols designated by the EPA as priority pollutants (293-295) were included. As shown in Table XI, specific guidelines for the maximum permissible concentration of many phenolic compounds in drinking water have been set by the EPA (1), the U.S.S.R. (1, 52) and Health and Welfare Canada (36). The Council on Environmental Pollutants (296) recently formulated a list of voluntary reference compounds which are to be used for comparison of improvements in new or modified techniques with existing methodology. Most of the reference compounds listed are known water pollutants for which present methods of isolation, separation, identification and quantitation are considered inadequate. As indicated in Table XI, seven of these thirteen consensus voluntary reference phenols were included in this study.

Acetic anhydride and propionic anhydride were used successfully for the preparation of acyl derivatives of all the phenols shown in Table XI. The direct acylation procedure for derivatization of trace phenolic concentrations was readily adaptable to both large and small sample volumes. Acylation proceeded rapidly to completion at room temperature using as little as 1 mL of acetic anhydride or propionic anhydride to derivatize a basified 1-litre water sample. The derivatives, once extracted into an organic solvent, were stable and

Table XI. Simple Phenolic Compounds Derivatized and Quantitated Using the Aqueous Acylation Method

Class	Representative Compounds		Water Quality Criteria (µg/L)		
	Number	Name	Health and Welfare Canada[1]	EPA[2]	U.S.S.R.[2]
Parent Compound	1	Phenol[3,4]	Less than 2 µg/L	0.05	1.0
Alkylphenols	2	o-Cresol		-[5]	30.0
	3	m-Cresol		-	-
	4	p-Cresol		-	-
	5	2,3-Dimethylphenol		-	-
	6	2,4-Dimethylphenol[3]		28.00	-
	7	2,5-Dimethylphenol		-	250
	8	2-Ethylphenol		-	-
	9	3-Ethylphenol		-	-
	10	4-Ethylphenol		-	-
	11	2-Isopropylphenol		-	-
	12	3-Isopropylphenol		-	-
	13	4-Isopropylphenol		-	-
	14	2-Hydroxyacetophenone		-	-
	15	3-Hydroxyacetophenone		-	-
	16	4-Hydroxyacetophenone		-	-
Methoxyphenols	17	Guaiacol[4]		-	-
	18	Euginol		-	-
	19	Isoeuginol		-	-
Polyhydric phenols	20	Catechol[4]		-	-

Table XI (continued)

Class	Number	Name	Health and Welfare Canada[1]	EPA[2]	U.S.S.R.[2]
Nitrophenols	21	2-Nitrophenol[3]	Less than 2 µg/L	250	60
	22	3-Nitrophenol		500	60
	23	4-Nitrophenol[3,4]		100	20
	24	3-Fluoromethyl-4-nitrophenol		-	-
Halophenols	25	2-Chlorophenol[3]		0.01	-
	26	4-Chlorophenol		1.00	-
	27	2,6-Dichlorophenol		0.02	2.0
	28	2,4-Dichlorophenol		0.02	2.0
	29	2,4,6-Trichlorophenol		0.40	0.40
	30	2,4,5-Trichlorophenol		0.40	0.40
	31	2,3,4,6-Tetrachlorophenol		1.00	-
	32	4,6-Dibromo-o-cresol		-	-
	33	2,3,4,5-Tetrachlorophenol		1.00	-
	34	Pentachlorophenol[3,4]		0.50	300
	35	4-Bromophenol		-	-
	36	6-Chlorothymol		-	-
	37	4-Chloro-3,5-dimethylphenol		-	-
	38	4-Chloro-3-methylphenol[3]		0.10	-
	39	4-Chloro-2-methylphenol		0.10	-
	40	4-Bromo-2,5-dichlorophenol		0.02	2.0
Polyaromatic phenols	41	1-Naphthol[4]		-	100

1. See reference number 36.
2. See reference number 1.
3. Included on the EPA List of 129 Priority Pollutants.
4. Consensus Voluntary Reference Compound.
5. Maximum acceptable concentration limits have not been set.

could be stored for as long as one week at 4 °C with virtually no decomposition. Results for the GLC analysis of each group of phenols listed in Table XI are now discussed in greater detail.

a) Nitrophenols and 1-Naphthol

Illustrated in Figure 7 are the differences in retention times of the original phenols and the corresponding acetylated derivatives when chromatographed on a 5% OV-101 column. The identities of the compounds giving rise to each peak in Figure 7 were confirmed by the mass spectral data shown in Table XII. Plausible mass spectral fragmentation pathways for acetate derivatives of NPs and 1-NAP are shown in Figure 8. The fragmentation pathways of underivatized 1-NAP and the NPs are similar to those of their respective acetate derivatives following the loss of the neutral molecule ketene ($O=C=CH_2$) from the molecular ion. The major fragment ions in each spectrum are consistent with literature reports on the fragmentation of phenols and aromatic nitro compounds (297). As shown in Table XII, the mass spectra obtained for 2-NP and 4-NP were very similar. The fragment ion m/z 122, which may be produced as a result of an "ortho" effect (Scheme 7), was absent from the mass spectrum of 4-NP.

The gas chromatogram of 2- and 4-nitrophenyl acetate and 1-naphthyl acetate (Figure 9) was obtained by adding 0.2 μmoles, 0.2 μmoles and 0.1 μmoles, respectively, of each phenol to 250 mL of distilled water and acetylating these phenols directly in

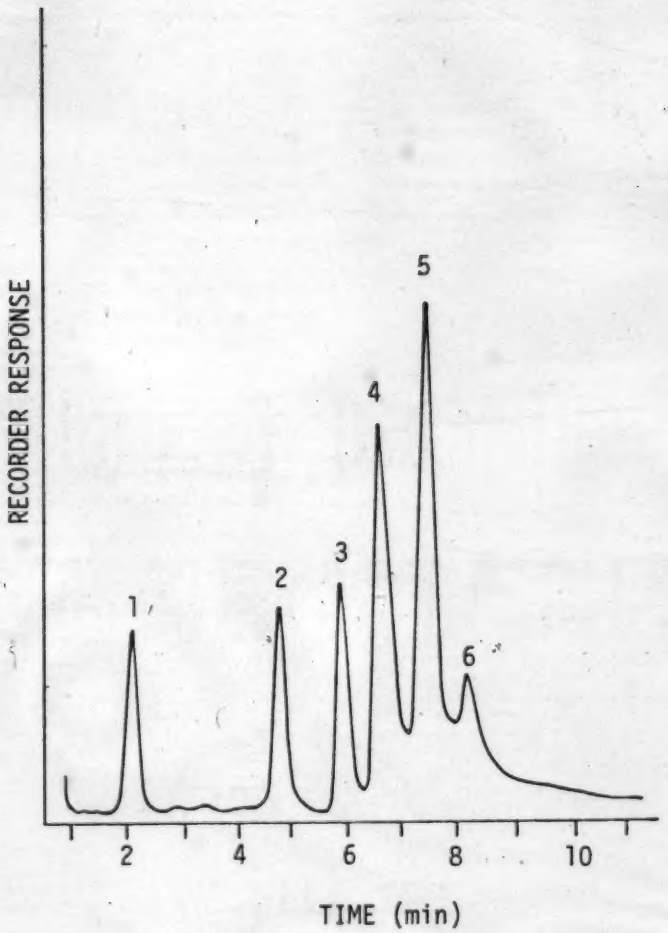


Figure 7. GLC-MS total ion trace of 2-nitrophenol, 4-nitrophenol, 1-naphthol and their respective acetate derivatives. Peak 1: 2-nitrophenol; 2: 2-nitrophenyl acetate; 3: 4-nitrophenyl acetate; 4: 1-naphthol; 5: 1-naphthyl acetate; 6: 4-nitrophenol. Chromatographic conditions: 5% OV-101, 100 - 220°C at 8°/min.

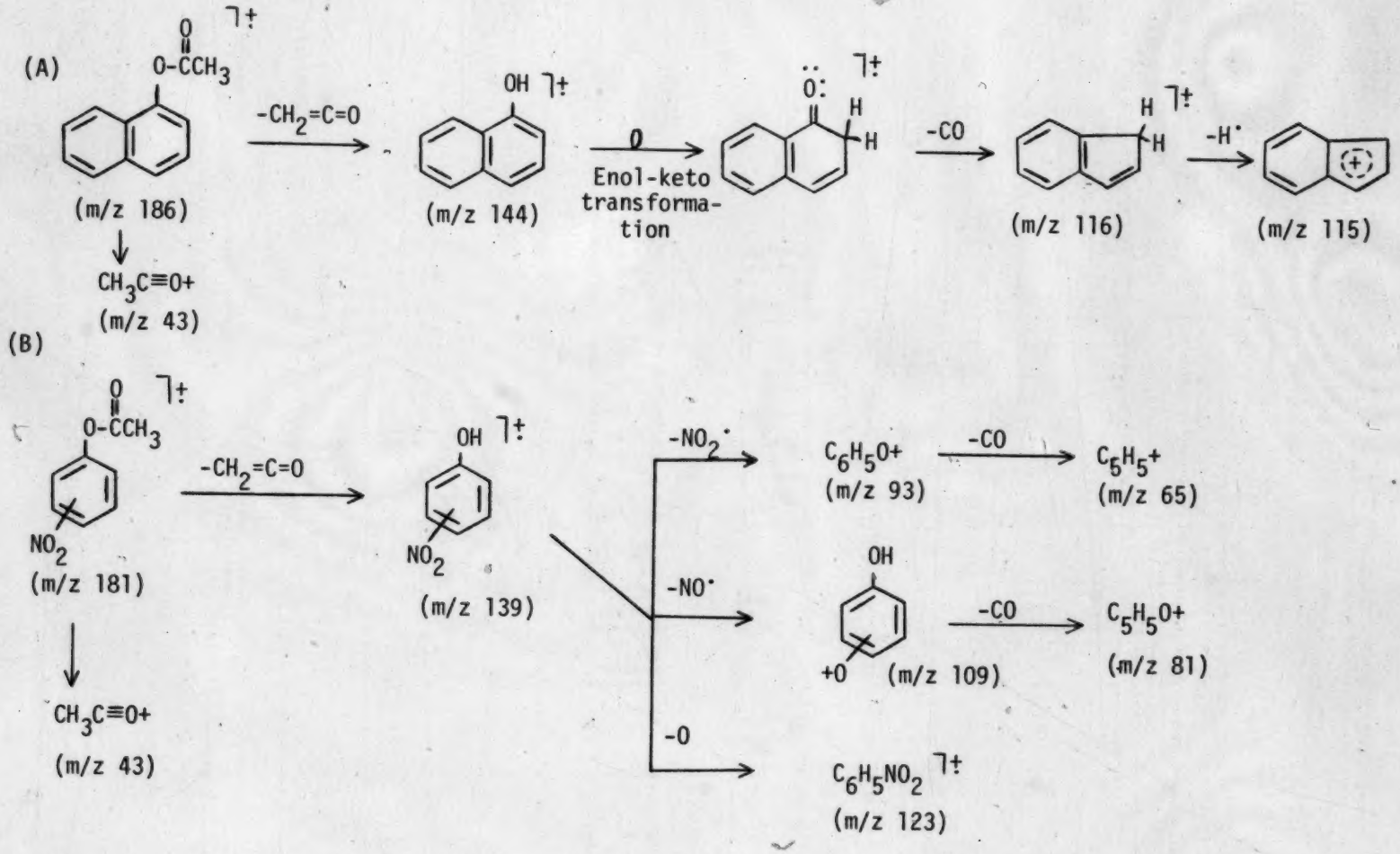
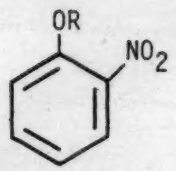
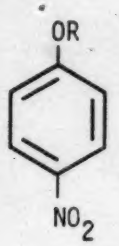


Figure 8. Mass spectral fragmentation of acetate derivatives of (A) 1-naphthol and (B) 2-, 3- and 4-nitrophenol.

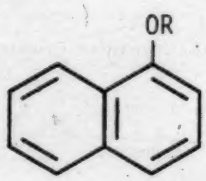
Table XII. Diagnostic Fragment Ions in the Mass Spectra of 2-nitrophenol (Ia), 4-nitrophenol (IIa), 1-naphthol (IIIa) and Their Respective Acetyl Derivatives, Ib, IIb and IIIb



I



II



III

I-III: a) R=H
b) R=$COCH_3$

Compound		
I	a	139(100), 122(5), 109(31), 81(21), 65(11), 63(10)
	b	181(17), 139(100), 123(5), 122(5), 109(20), 81(10), 65(6), 63(12), 43(16)
II	a	139(100), 123(6), 109(55), 65(21), 63(8)
	b	181(100), 139(77), 123(27), 109(79), 93(20), 81(18), 65(10), 43(21)
III	a	144(100), 116(53), 115(99), 89(11)
	b	186(21), 144(100), 116(35), 115(65), 43(2)

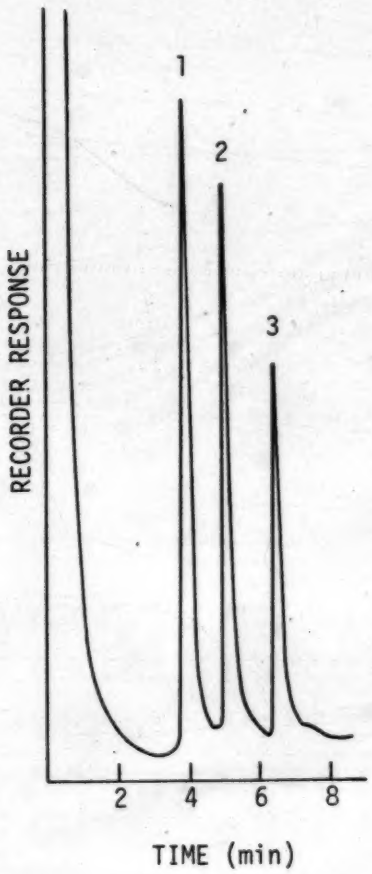
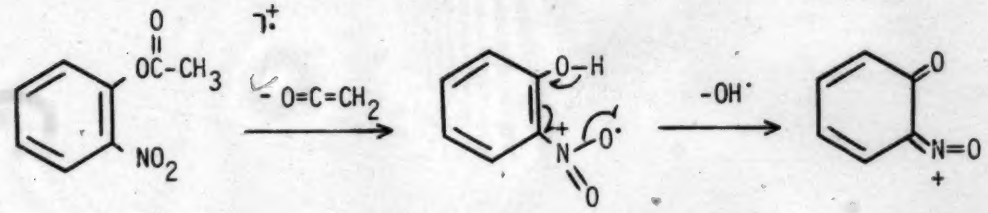


Figure 9. Gas-liquid chromatographic separation of acetate derivatives of 2-nitrophenol (1), 4-nitrophenol (2) and 1-naphthol (3) prepared directly in aqueous solution. A 250 mL water sample containing 0.2 μmoles of each nitrophenol and 0.1 μmole of 1-naphthol was acetylated and extracted. Chromatographic conditions: 5% OV-101, 100 - 220°C at 8°/min.



Scheme 7.

the aqueous solution. The conversion was quantitative. Only the acetate esters were detected by GLC; peaks corresponding to the underivatized phenols were absent from the trace.

Calibration graphs (Figure 10) were similarly obtained by GLC analysis of methylene chloride extracts of acetylated 250 mL aqueous solutions containing 0.02-0.2 μmoles each of 2- and 4-NP and 0.1 μmoles of internal standard 1-NAP. When 250 mL solutions containing 0.045-0.18 μmoles each of 2- and 4-NP plus 0.1 μmole of 1-NAP were analyzed by this procedure, recoveries of 97% or greater were repeatedly obtained. Detection of all three compounds at concentrations as low as 1 μg/L (Figure 11) was possible using FID. This compares favorably with other

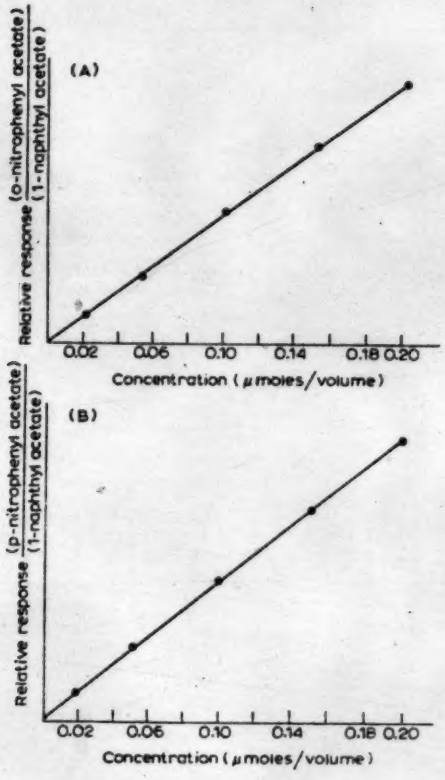


Figure 10. Calibration graphs for the acetate derivatives of (A) 2-nitrophenol and (B) 4-nitrophenol in the concentration range 0.02-0.2 μmoles in 250 mL distilled water. Chromatographic conditions: 5% OV-101, 100 - 220°C at 8°/min.

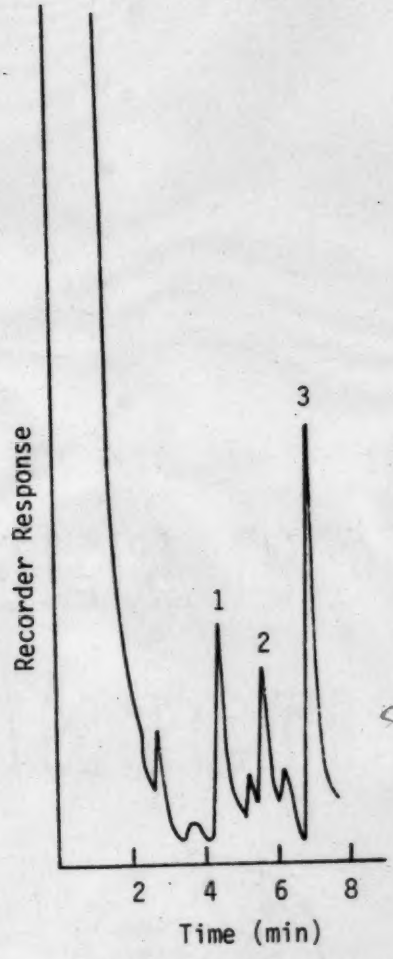


Figure 11. A flame ionization detector could easily detect as little as 1 µg/L each of 2- and 4-nitrophenol and 1-naphthol in a 500 mL water sample following aqueous acetylation. Peak 1: 2-nitrophenyl acetate; 2: 4-nitrophenyl acetate; 3: 1-naphthyl acetate. Chromatographic conditions: 5% OV-101, 100-220°C at 8°/min.

procedures for the detection of 4-NP (138) and 1-NAP (188) using ECD-GLC.

Many underivatized phenolic compounds can be analyzed gas chromatographically using acid deactivated columns which overcome peak tailing and adsorption; 4-NP is an exception. Shackelford and Webb (87) compared the performance of SP-2250, SP-1000, Ultra-Bond, SP-1240 DA and Tenax GC for the GLC analysis of underivatized phenols. 4-NP did not elute from SP-2250 and the other four columns gave very poor results for the chromatography of injected concentrations of less than 100 ng. In contrast, the stable acetate derivative of 4-NP prepared in this study has excellent GLC properties even using a conventional undeactivated liquid phase such as OV-101.

b) Alkyl-, Methoxy- and Polyhydric Phenols

Illustrated in Figure 12 is the gas chromatographic separation of twelve alkyl-, methoxy-, polyhydric and nitrophenols using a packed 1% SP-1240 DA column with 4,6-DBC as internal standard. As shown in Table XIII, however, many ethyl-, dimethyl- and isopropylphenols closely overlap using this column and the isomer pairs m- and p-CRE, 3- and 4-ethylphenol (EtP) and 2,4- and 2,5-DMP could not be resolved. Similar results were obtained using a packed 5% OV-101 column. A gas chromatogram of 2-, 3- and 4-ethylphenyl acetates on OV-101 is illustrated in Figure 13; alterations in temperature programming and flow rate did little to improve the resolution achieved.

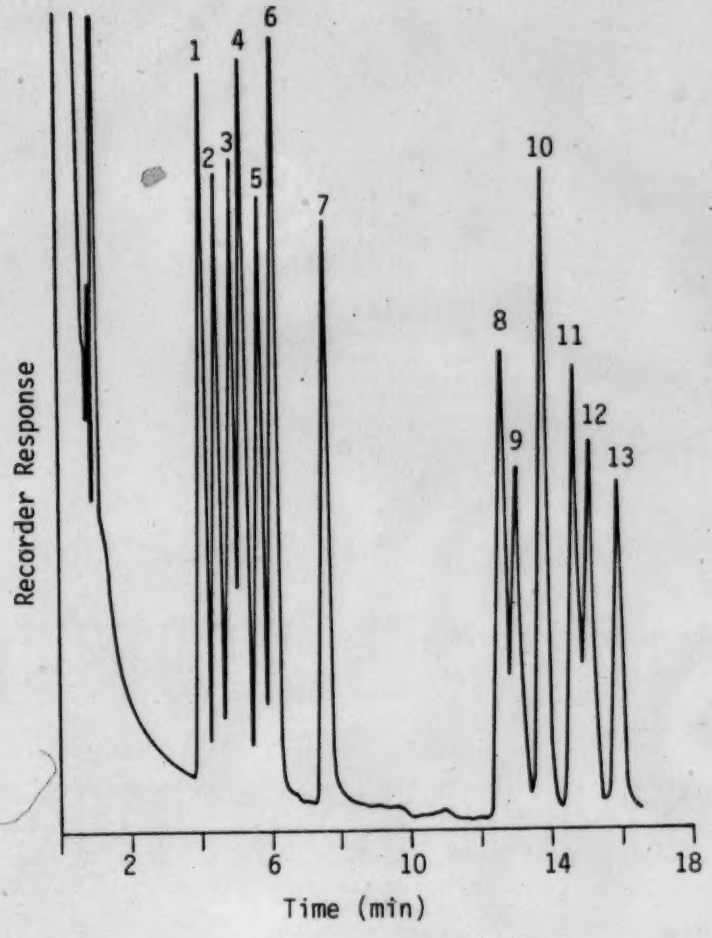


Figure 12. Gas-liquid chromatographic separation of a mixture of alkyl-, methoxy- and nitrophenols following aqueous derivatization with acetic anhydride. Peaks are acetate esters of 1: 2-ethylphenol; 2: 2-isopropylphenol; 3: 2,4-dimethylphenol; 4: 4-ethylphenol; 5: 2,3-dimethylphenol; 6: 4-isopropylphenol; 7: guaiacol; 8: catechol; 9: 2-nitrophenol; 10: eugenol; 11: 4,6-dibromo-o-cresol (internal standard); 12: 3-nitrophenol; 13: 4-nitrophenol. Chromatographic conditions: 1% SP-1240 DA, 75-170°C at 4°/min.

Table XIII. GLC Separation of Alkylphenol Isomers Using Packed Columns

Phenol (As acetate derivative)	Relative Retention Time		
	5% OV-101[1]	0.1% SP-1000[2]	1% SP-1240 DA[3]
phenol	0.46	0.13	0.20
o-cresol	0.61	0.31	0.26
m-cresol	0.68	0.41	0.31
p-cresol	0.68	0.48	0.31
2-ethylphenol	0.77	0.51	0.33
3-ethylphenol	0.86	0.70	0.39
4-ethylphenol	0.89	0.83	0.41
2,3-dimethylphenol	0.89	1.11	0.44
2,4-dimethylphenol	0.83	1.10	0.39
2,5-dimethylphenol	0.82	1.07	0.37
2-isopropylphenol	-	-	0.36
3-isopropylphenol	-	-	0.45
4-isopropylphenol	-	-	0.47
4-bromophenol	1.0	1.0	-
4,6-dibromo-o-cresol	-	-	1.0

Column temperatures: 1. 75 - 220°, 8°/min
2. 210°, isothermal
3. 75 - 170°, 4°/min

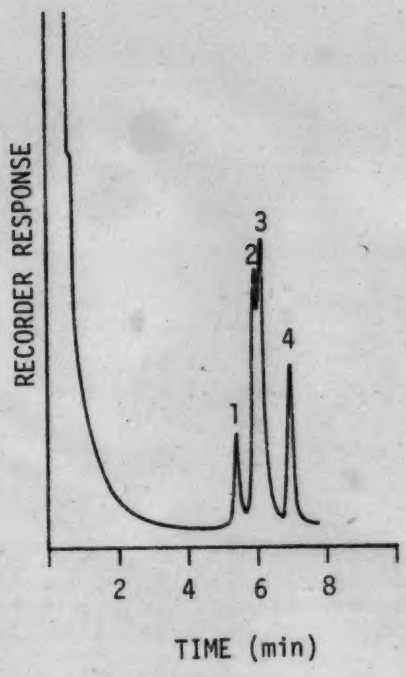
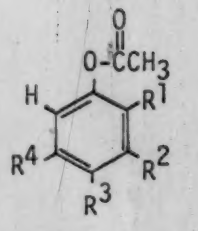


Figure 13. Complete resolution of acetate derivatives of 2-, 3- and 4-ethylphenol could not be achieved using a packed 5% OV-101 column. Peaks are acetate esters of 1: 2-ethylphenol; 2: 3-ethylphenol; 3: 4-ethylphenol; 4: 4-bromophenol (internal standard). Chromatographic conditions: 75-220°C at 8°/min.

Diagnostic fragment ions in the mass spectra of acetate derivatives of some alkyl-, methoxy- and polyhydric phenols are summarized in Table XIV. General fragmentation pathways of acetate ester derivatives of CREs, DMPs and EtPs are shown in Figure 14. The mass spectra of all of the acetylated phenols exhibit an intense (M-42) fragment ion due to the loss of a neutral molecule of ketene from the molecular ion. The cresols contain a strong (M-43) ion, possibly due to the formation of a stable hydroxytropylium ion. The intensity of the (M-43) ion in the spectra of DMPs and EtPs is reduced at the expense of a strong m/z 107 fragment ion due to further loss of a methyl radical. The ions m/z 91, m/z 77 and m/z 65, which correspond to fragments $C_7H_7^+$, $C_6H_5^+$ and $C_5H_5^+$, are commonly encountered in the mass spectra of alkylbenzenes and alkylphenols. Ions of similar molecular weight occur both in the mass spectra of DMPs and EtPs. Since the fragments are structural isomers, the DMPs and EtPs could not be distinguished by the mass spectral data shown in Table XIV.

It has been demonstrated (161) that 0.1% SP-1000 resolves the three cresol isomers. Other alkylphenols have also been separated into the sequential groups of phenol, cresols, ethylphenols and dimethylphenols with no overlap between the groups. As shown in Table XIII, similar results were achieved for the acetate ester derivatives of the alkylphenols listed. During the course of this study, the SP-1000 column was found to be most useful for the identification of cresol isomers. As

Table XIV. Diagnostic Fragment Ions in the Mass Spectra of Acetate Derivatives of Some Alkyl-, Methoxy- and Polyhydric Phenols



Name of Phenol	Structure of Acetate Derivative R^1	R^2	R^3	R^4	m/z (% Relative Abundance)
o-cresol	$-CH_3$	-H	-H	-H	150[M⁺](21), 108(100), 107(46), 43(21)
p-cresol	-H	-H	$-CH_3$	-H	150[M⁺](12), 108(100), 107(57), 43(13)
2,3-dimethylphenol	$-CH_3$	$-CH_3$	-H	-H	164[M⁺](14), 122(100), 121(20), 107(92), 91(30), 77(35), 65(10), 43(20)
2,4-dimethylphenol	$-CH_3$	-H	$-CH_3$	-H	164[M⁺](9), 122(100), 121(23), 107(72), 91(21), 77(22), 65(5), 43(8)
2,5-dimethylphenol	$-CH_3$	-H	-H	$-CH_3$	164[M⁺](12), 122(100), 121(23), 107(85), 91(45), 77(53), 65(20), 43(42)
2-ethylphenol	$-C_2H_5$	-H	-H	-H	164[M⁺](8), 122(52), 121(4), 107(100), 91(13), 77(23), 43(12)
3-ethylphenol	-H	$-C_2H_5$	-H	-H	164[M⁺](12), 122(84), 107(100), 91(10), 77(16), 43(5)
4-ethylphenol	-H	-H	$-C_2H_5$	-H	164[M⁺](5), 122(36), 121(5), 107(100), 91(18), 77(31), 43(23)
2-isopropylphenol	$-C_3H_7$	-H	-H	-H	178[M⁺](6), 136(28), 122(12), 121(100), 91(45), 77(29), 43(49)
3-isopropylphenol	-H	$-C_3H_7$	-H	-H	178[M⁺](8), 136(54), 122(10), 121(100), 91(21), 77(11), 43(18)
4-isopropylphenol	-H	-H	$-C_3H_7$	-H	178[M⁺](4), 136(24), 122(14), 121(100), 91(35), 77(21), 43(21)
guaiacol	$-OCH_3$	-H	-H	-H	166[M⁺](7), 124(100), 109(82), 81(17), 43(5)
eugenol	$-OCH_3$	-H	$-CH_2CH=CH_2$	-H	206[M⁺](7), 164(100), 149(34), 43(5)
catechol	$-O\overset{O}{\overset{\|}{C}}CH_3$	-H	-H	-H	194[M⁺](3), 152(14), 110(100), 43(61)

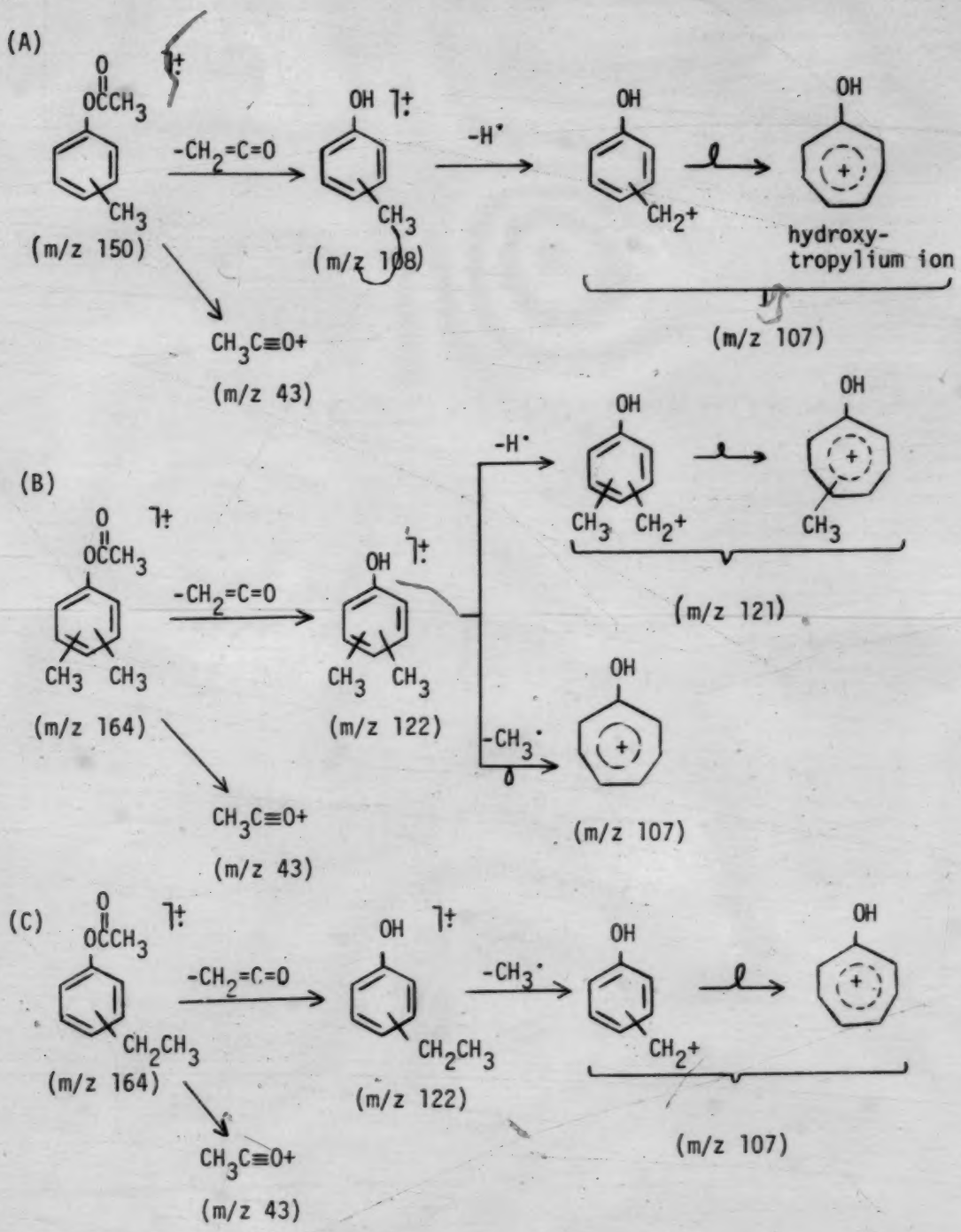


Figure 14. General mass spectral fragmentation pathways of acetate ester derivatives of (A) cresols, (B) dimethylphenols and (C) ethylphenols.

illustrated in Figure 15, the cresyl acetate derivatives could be easily separated within 10 min. SP-1000 packing material is thermally unstable, with an upper temperature limit of 225 °C (161). The column was, therefore, unsuitable for the analysis of alkylphenols less volatile than the cresols. At 210 °C, the long retention times and broad peaks obtained for acetylated DMPs and EtPs resulted in low detection sensitivities for these compounds. The dimethylphenyl acetate isomers could not be resolved using SP-1000.

In Figure 16 are depicted calibration graphs for acetylated PHE, o-CRE and p-CRE in distilled water which were linear over the 0.01-0.40 μmoles/volume concentration range using an OV-101 column and FID-GLC. The gas chromatogram in Figure 17 was obtained by acetylating 0.01 μmoles of PHE, o- and p-CRE and 0.10 μmoles of 4-BrP directly in 1 L of distilled water. The detection limit of these three compounds on an OV-101 column was 1 nmole/L (0.1 μg/L) using FID-GLC. Despite these excellent detection sensitivities, unequivocal identification and quantitation of complex mixtures could not be achieved because many alkylphenol compounds and isomers within each group of compounds could not be separated. The resolution of complex mixtures of acylated alkylphenols would require the use of high efficiency capillary columns.

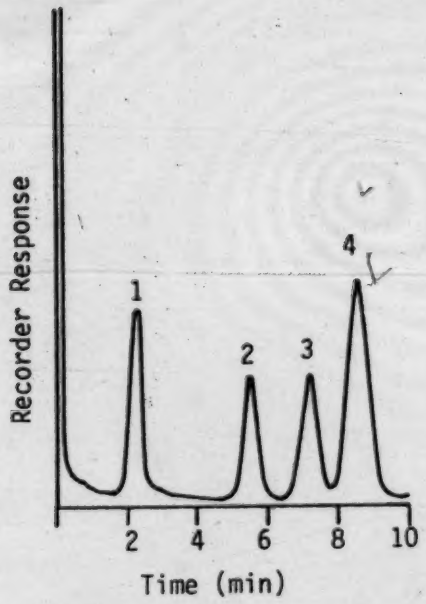


Figure 15. Separation of acetate derivatives of phenol (1), o-cresol (2), m-cresol (3) and p-cresol (4) using a Carbopack C/0.1% SP-1000 column. Chromatographic conditions: Isothermal at 210°C.

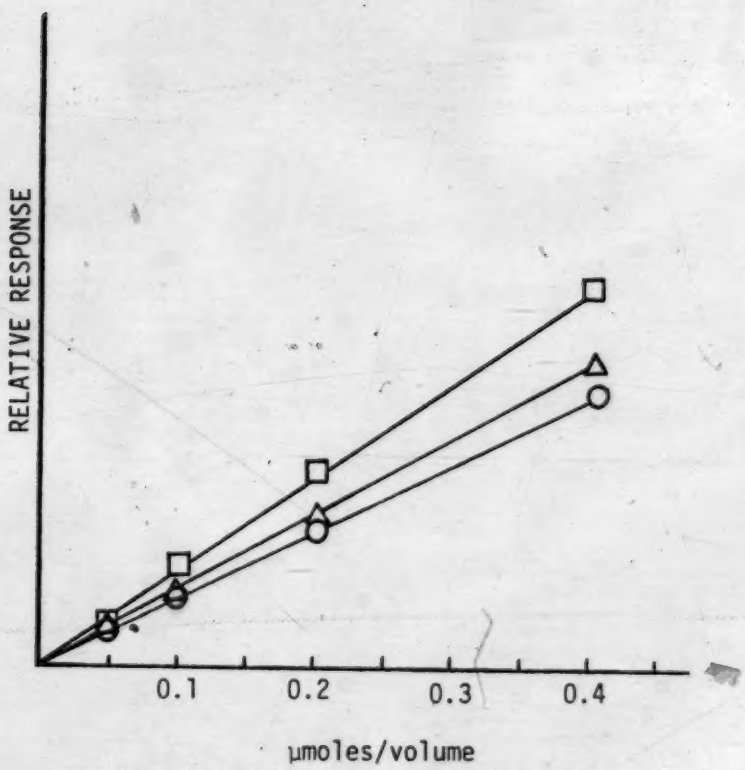


Figure 16. Calibration graphs for the acetate derivatives of phenol (Δ), o-cresol (O) and p-cresol (□) in the concentration range 0.01-0.4 µmoles/volume. Chromatographic conditions: 5% OV-101, 75-220°C at 8°/min. 4-Bromophenol (0.1 µmole/volume) was used as the internal standard for calibration.

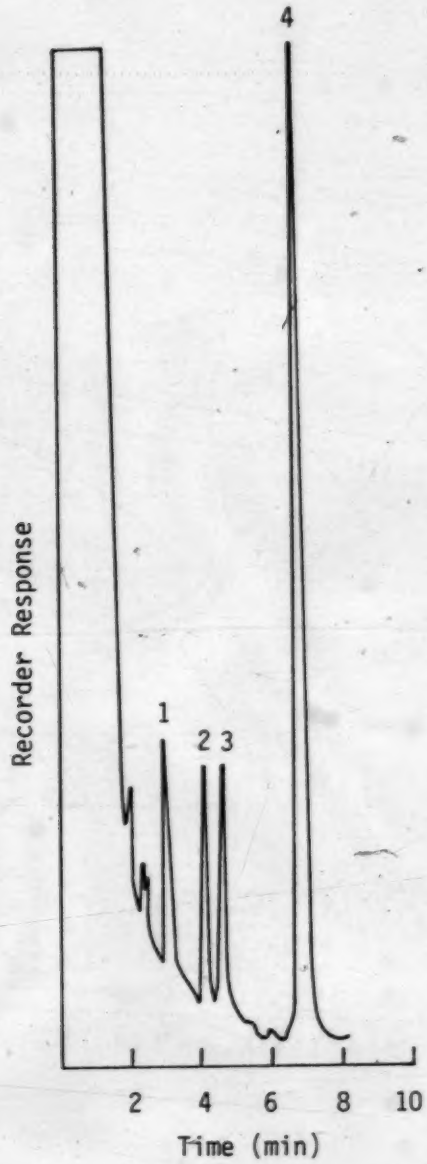
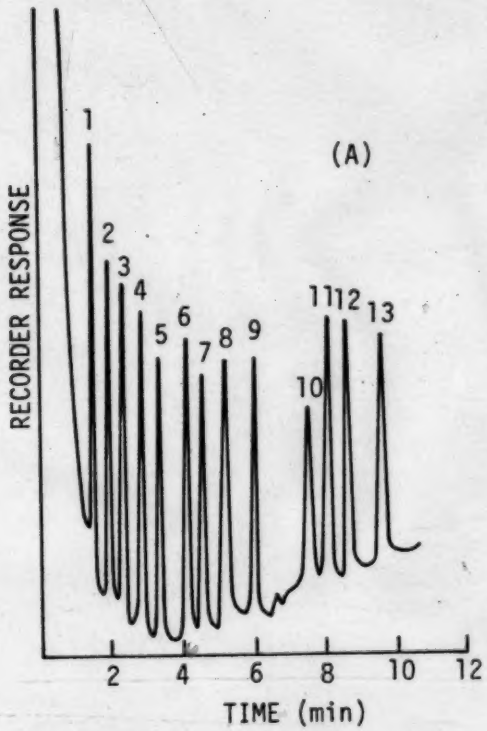


Figure 17. Gas-liquid chromatogram obtained by direct aqueous acetylation of 0.01 μmoles each of phenol, o-cresol and p-cresol and 0.10 μmoles of internal standard 4-bromophenol in a 1 L distilled water sample. Peak 1: phenyl acetate; 2: o-cresyl acetate; 3: p-cresyl acetate; 4: 4-bromophenyl acetate. Chromatographic conditions: 5% OV-101, 75-220°C at 8°/min.

c) Chlorophenols (Selected Ion Monitoring-Mass Spectrometry)

Many gas chromatographic methods exist for quantitation of chlorinated phenols in biological fluids (72-75, 167), tissues (89, 98, 107, 170), river water (89, 197), industrial wastes (91, 108) and many other sample matrices (93, 101, 103, 104). A major limitation of these analytical techniques is the high degree of uncertainty associated with identification of compounds based simply on the coincidence of GLC retention times with those of authentic standards. Quantitation of chlorophenols at ppb levels has been frequently reported (72, 74, 75); natural constituents in a biological extract may, however, have GLC retention times similar to the chlorophenols of interest and the reliability of trace analysis using ECD-GLC has recently been questioned (6). Although ECD is highly sensitive, it is nonspecific and additional evidence is necessary to confirm the identities of GLC peaks. Confirmation of identity can be obtained by using the mass spectrometer as a very specific and sensitive detector in selected ion monitoring (SIM) mode (178, 197, 298).

In the present study, both propionate and acetate derivatives of PHE, o-CRE, p-CRE, 2-MCP, 4-MCP, 2,4-DCP, 2,6-DCP, 2,4,6-TCP, 2,4,5-TCP, 2,3,4,5-TeCP, 2,3,4,6-TeCP, PCP and the internal standard 4,6-DBC were prepared directly in aqueous solution using the appropriate reagent anhydride. Typical chromatograms of the acetates and propionates on 1% SP-1240 DA are shown in Figure 18; complete resolution was



Peak identification, acyl derivatives of:

1. phenol
2. o-cresol
3. p-cresol
4. 2-chlorophenol
5. 4-chlorophenol
6. 2,6-dichlorophenol
7. 2,4-dichlorophenol
8. 2,4,6-trichlorophenol
9. 2,4,5-trichlorophenol
10. 2,3,4,6-tetrachlorophenol
11. 4,6-dibromo-o-cresol
12. 2,3,4,5-tetrachlorophenol
13. pentachlorophenol

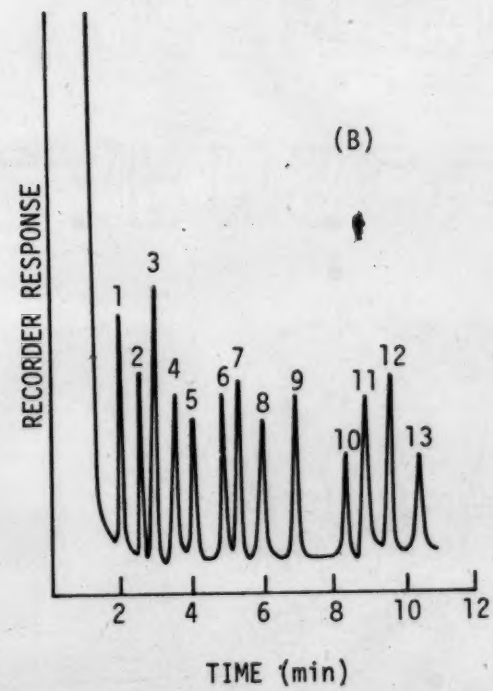


Figure 18. Gas chromatograms obtained by derivatization of 20 nmoles of each phenol in 100 mL distilled water with acetic anhydride (A) and propionic anhydride (B). Chromatographic conditions: 1% SP-1240 DA, 75-170°C at 8°/min.

achieved within a 12 min period. Similar resolution could also be achieved using conventional column packings such as OV-101 (Figure 19). Identities of the compounds giving rise to each peak in Figure 18 were confirmed by the mass spectral data shown in Table XV. Following the expulsion of ketene or methylketene, respectively, from the molecular ions of the acetate and propionate esters, the mass spectra of the two phenol derivatives shown in Table XV are very similar. Plausible fragmentation pathways which explain the major ions in the mass spectra of the propionate and acetate derivatives of 4,6-DBC and PCP are shown in Figure 20 and Figure 21, respectively. The major mass spectral fragmentation pathway of aromatic chlorides and bromides (297) is due to the loss of a halogen radical, unless alkyl substituents larger than methyl are present. In such compounds, the loss of the halogen radical is more pronounced than the loss of a hydrogen halide molecule. As shown in Figure 20, the m/z 187 (m/z 185) ion cluster is due to the loss of a bromine radical from the odd electron 4,6-DBC ion.

In Table XV, the chloro- and bromophenols listed often have several ions designated as the molecular ion ([M^{+}]). Both bromine (Br^{79}, Br^{81}) and chlorine (Cl^{35}, Cl^{37}) have two natural isotopes which occur with appreciable abundance. These isotopes result in the very characteristic clusters of ions separated by two mass units which are prevalent in the mass spectra of the halogenated phenols included in Table XV. Diagnostic and

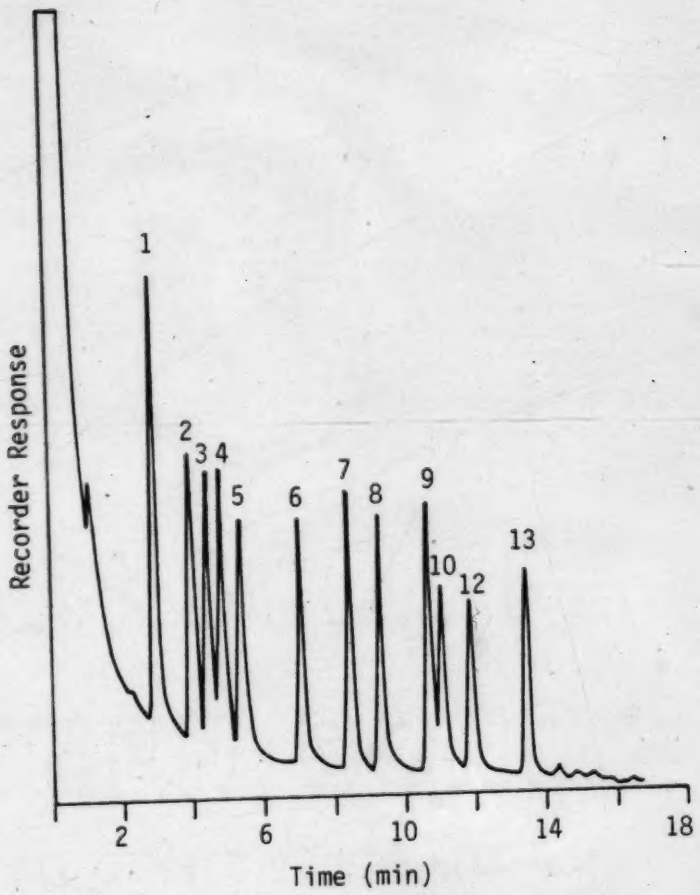
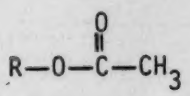
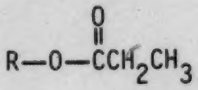


Figure 19. Acetate derivatives of chlorophenols can also be resolved using a packed 5% OV-101 column. Chromatographic conditions: 75-220°C at 8°/min.

Table XV. Diagnostic Fragment Ions in the Mass Spectra (70 eV ion source) of the Acetyl (I) and Propionyl (II) Derivatives of Environmental Phenols



I



II

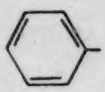
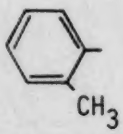
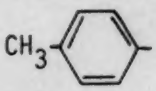
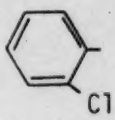
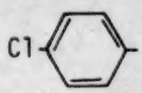
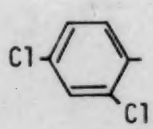
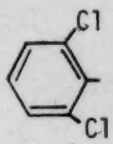
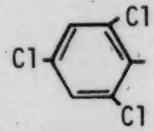
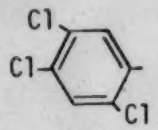
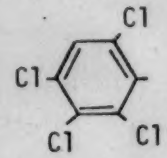
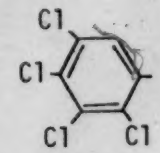
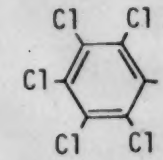
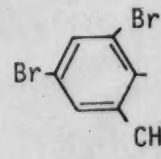
Compound	R	m/z (% Relative Abundance)
phenol		I 136[M^{+}](12), 94(100), 43(6) II 150[M^{+}](15), 95(7.7), 94(100), 57(21)
o-cresol		I 150[M^{+}](21), 108(100), 107(46), 43(21) II 164[M^{+}](16), 109(10), 108(100), 107(25), 57(87)
p-cresol		I 150[M^{+}](12), 108(100), 107(57), 43(13) II 164[M^{+}](9), 109(8), 108(100), 107(31), 57(18)
2-chlorophenol		I 170[M^{+}](9), 130(31), 128(100), 43(13) II 186[M^{+}](8), 184[M^{+}](20), 130(33), 129(10), 128(100), 57(89)
4-chlorophenol		I 170[M^{+}](10), 130(34), 128(100), 43(10) II 186[M^{+}](6), 184[M^{+}](16), 130(32), 128(100), 57(45)
2,4-dichlorophenol		I 206[M^{+}](11), 204[M^{+}](18), 164(60), 162(100), 43(18) II 220[M^{+}](8), 218[M^{+}](10), 166(5), 164(18), 162(28), 57(100)
2,6-dichlorophenol		I 206[M^{+}](7), 204[M^{+}](11), 164(63), 162(100), 43(13) II 220[M^{+}](9), 218[M^{+}](11), 166(9), 164(40), 162(63), 57(100)
2,4,6-trichloro-phenol		I 242[M^{+}](4), 240[M^{+}](14), 238[M^{+}](14), 200(30), 198(100), 196(97), 43(25) II 256[M^{+}](9), 254[M^{+}](31), 252[M^{+}](31), 200(30), 198(92), 196(100), 57(84)

Table XV (continued):

Compound	R	m/z (% Relative Abundance)
2,4,5-trichloro-phenol		I 242[M⁺](3), 240[M⁺](12), 238[M⁺](12), 200(33), 198(99), 196(100), 43(28) II 256[M⁺] (6), 254[M⁺](17), 252[M⁺] (17), 200(31), 198(98), 196(100), 57(38)
2,3,4,6-tetra-chlorophenol		I 276[M⁺](5), 274[M⁺](12), 272[M⁺](9), 234(47), 232(100), 230(77), 43(25) II 290[M⁺](16), 288[M⁺](30), 286[M⁺] (25), 234(49), 232(90), 230(72), 57(100)
2,3,4,5-tetra-chlorophenol		I 276[M⁺](4), 274[M⁺](10), 234(49), 232(100), 230(82), 43(26) II 290[M⁺](5), 288[M⁺](12), 286[M⁺] (9), 234(33), 232(65), 230(59), 57(100)
pentachloro-phenol		I 312[M⁺](3), 310[M⁺](12), 308[M⁺] (17), 306[M⁺](11), 270(21), 268(59), 266(100), 264(67), 167(22), 165(22), 43(20) II 324[M⁺](12), 322[M⁺](18), 320[M⁺] (12), 270(7), 268(25), 266(38), 264(24), 169(12), 167(38), 165(38), 57(100)
4,6-dibromo-o-cresol		I 310[M⁺](5), 308[M⁺](10), 306[M⁺] (5), 268(52), 266(100), 264(54), 187(25), 185(25), 43(20) II 322[M⁺](10), 320[M⁺](7), 268(38), 266(80), 264(41), 187(18), 185(19), 57(100)

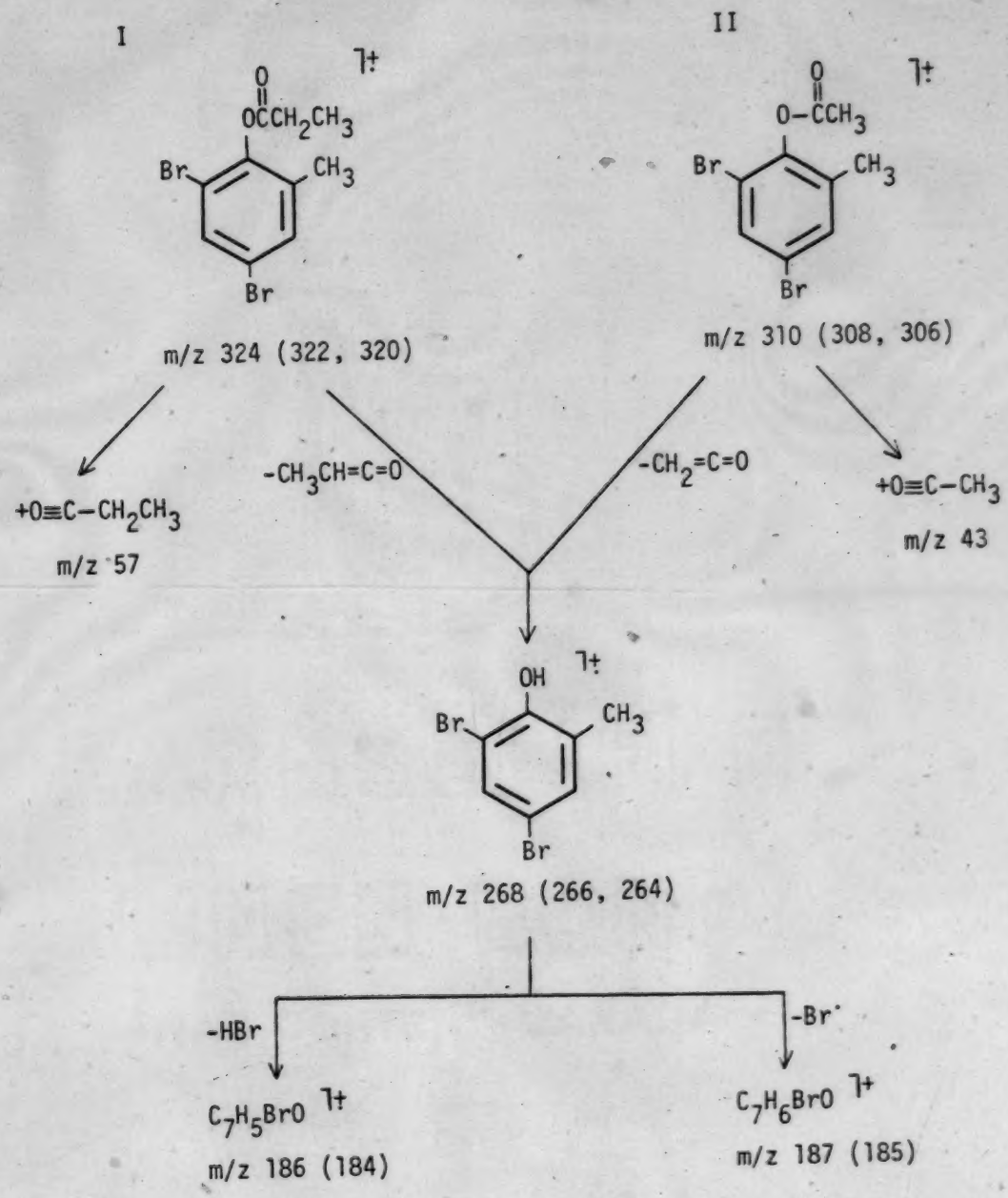


Figure 20. Mass spectral fragmentation pathways of the propionate (I) and acetate (II) ester derivatives of 4,6-dibromo-o-cresol.

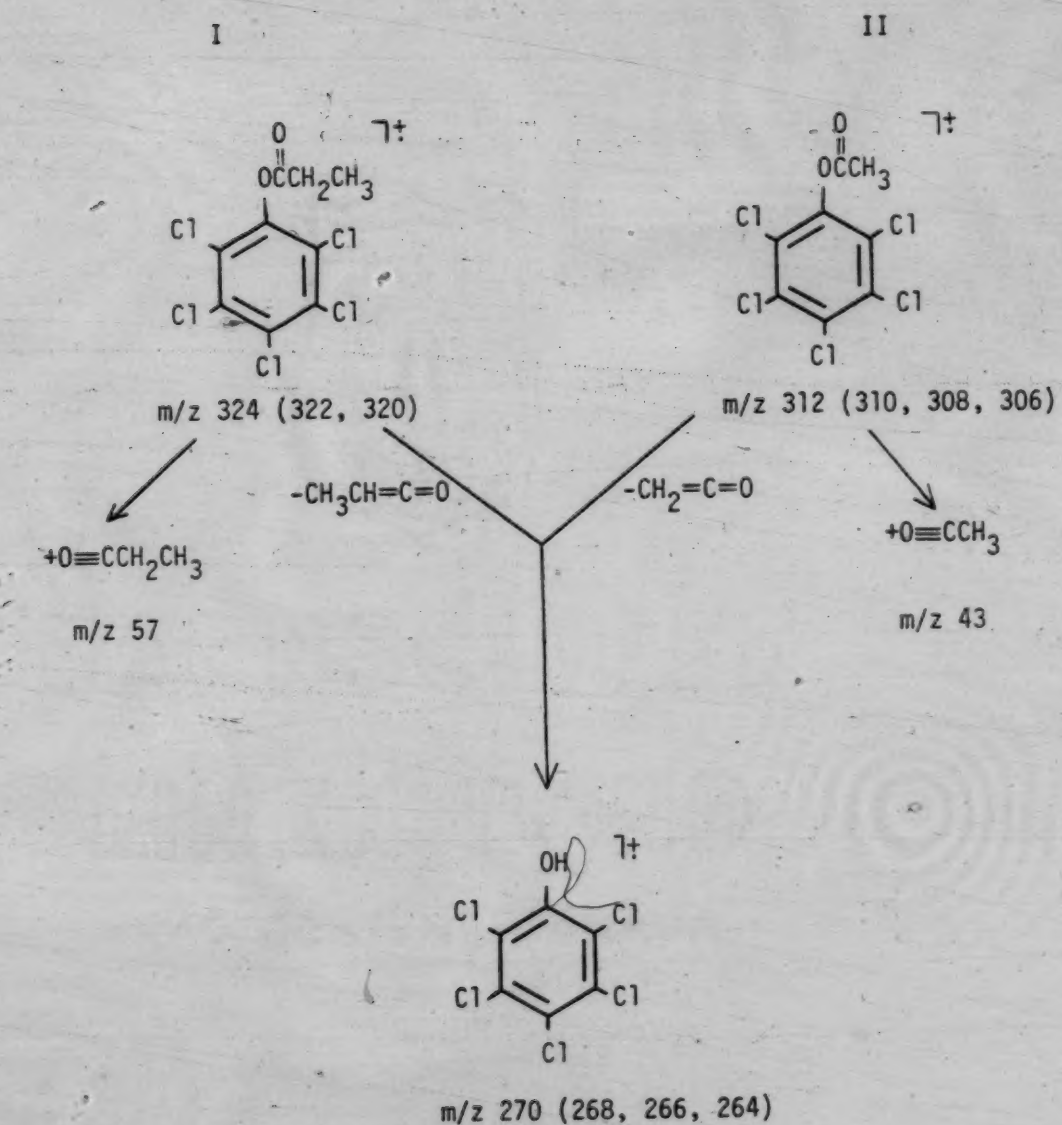


Figure 21. Mass spectral fragmentation pathways of the propionate (I) and acetate (II) ester derivatives of pentachlorophenol.

abundant ions were chosen from the mass spectral data shown in Table XV for SIM analysis of each of the thirteen phenols as acetate and propionate derivatives. The Hewlett Packard Model 5934A MS data system is capable of scanning twenty selected masses during a single run. A group of four masses was monitored at any one time and a total of five groups were selected for each SIM analysis. The ions chosen for SIM analysis of each phenyl acetate and phenyl propionate are shown in Tables XVI and XVII, respectively, and the SIM profiles obtained for standard mixtures of the thirteen phenols as acetate and propionate esters are shown in Figure 22 and Figure 23, respectively. The standard profiles were prepared by derivatizing 10 nmoles of each phenol in 5 mL of distilled water. In addition to the ions monitored in Figure 22 and Figure 23, other ions from Table XV could also be used for SIM. The abundance of the ions scanned could be optimized by changing the ion source voltage. As shown in Figure 24, the relative abundance of fragment ions in the mass spectrum of 2,4-dichlorophenyl propionate were affected by changes in ion source voltage. As the voltage decreased fewer low molecular weight fragments were produced and the abundance of the molecular ion simultaneously increased. In this study, optimum sensitivity for each phenol was achieved by using the ionization voltage at which the chosen screening ions were present at their maximal intensity.

Table XVI. Diagnostic Ions Used for Selected Ion Monitoring-Mass Spectrometry of Phenols Derivatized with Acetic Anhydride

Peak Number	Acetate Derivatives of:	Group	Ion (m/z)
1	phenol	I	94
2	o-cresol		108
3	p-cresol		108
4	2-chlorophenol		128, 130
5	4-chlorophenol		128, 130
6	2,6-dichlorophenol	II	162, 164, 204, 206
7	2,4-dichlorophenol		162, 164, 204, 206
8	2,4,6-trichlorophenol	III	196, 198, 200, 240
9	2,4,5-trichlorophenol		196, 198, 200, 240
10	2,3,4,6-tetrachlorophenol	IV	230, 232
11	4,6-dibromo-o-cresol		264, 266
12	2,3,4,5-tetrachlorophenol		230, 232
13	pentachlorophenol	V	264, 266, 268, 270

Table XVII. Diagnostic Ions Used for Selected Ion Monitoring-Mass Spectrometry of Phenols Derivatized with Propionic Anhydride

Peak Number	Propionate Derivatives of:	Group	Monitored Ions (m/z)
4	2-chlorophenol	I	128, 130, 184, 186
5	4-chlorophenol		128, 130, 184, 186
6	2,6-dichlorophenol	II	162, 164, 218, 220
7	2,4-dichlorophenol		162, 164, 218, 220
8	2,4,6-trichlorophenol	III	196, 198, 252, 254
9	2,4,5-trichlorophenol		196, 198, 252, 254
10	2,3,4,6-tetrachlorophenol	IV	230, 232, 288
11	4,6-dibromo-o-cresol		266
12	2,3,4,5-tetrachlorophenol		230, 232, 288
13	pentachlorophenol	V	264, 266, 268, 322

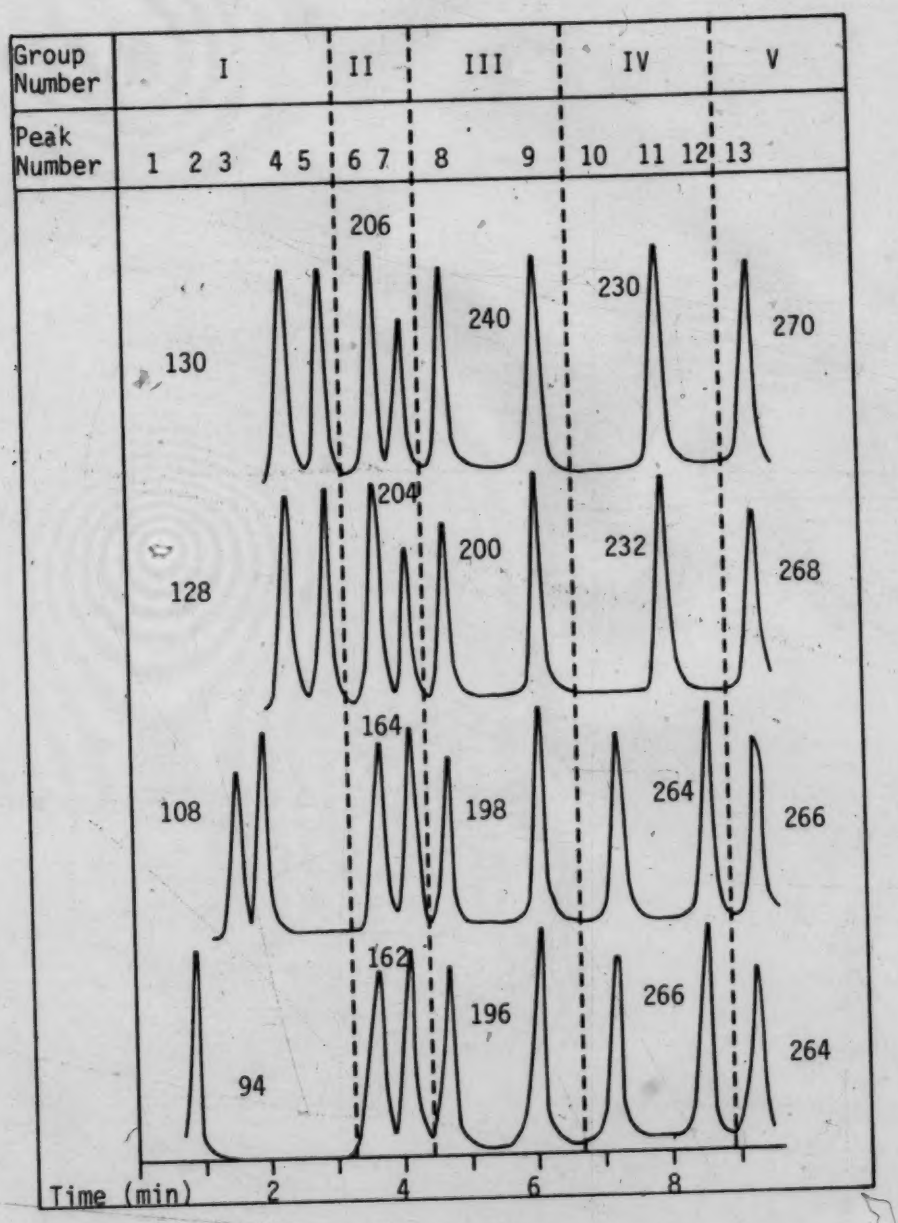


Figure 22. SIM-MS chromatogram of acetate derivatives of a standard mixture of thirteen phenolic compounds. Ion groups and the phenols corresponding to each peak number are identified in Table XVI. Chromatographic conditions: 1% SP-1240 DA, 75-170°C at 8°/min.

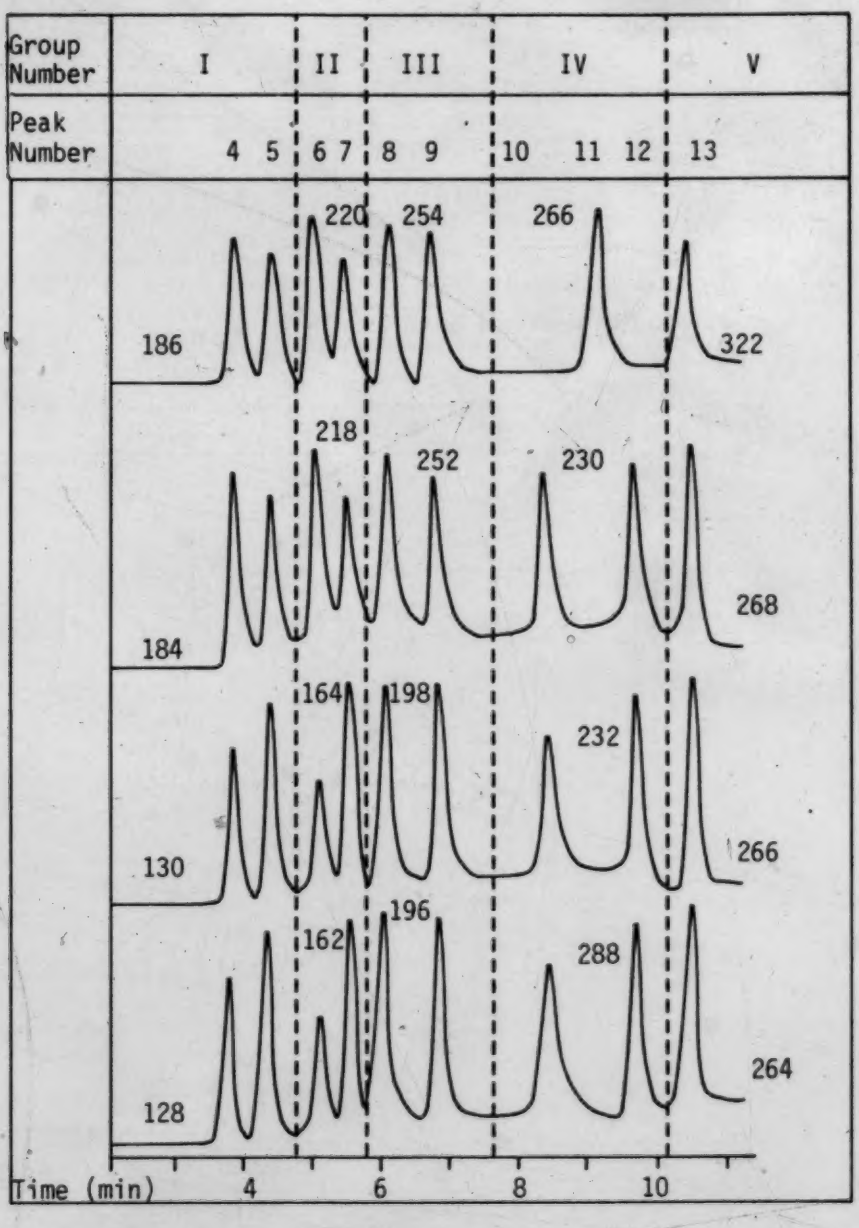


Figure 23. SIM-MS chromatogram of propionate derivatives of a standard mixture of ten phenolic compounds. Ion groups and the phenols corresponding to each peak number are identified in Table XVII. Chromatographic conditions: 1% SP-1240 DA, 75-170°C at 8°/min.

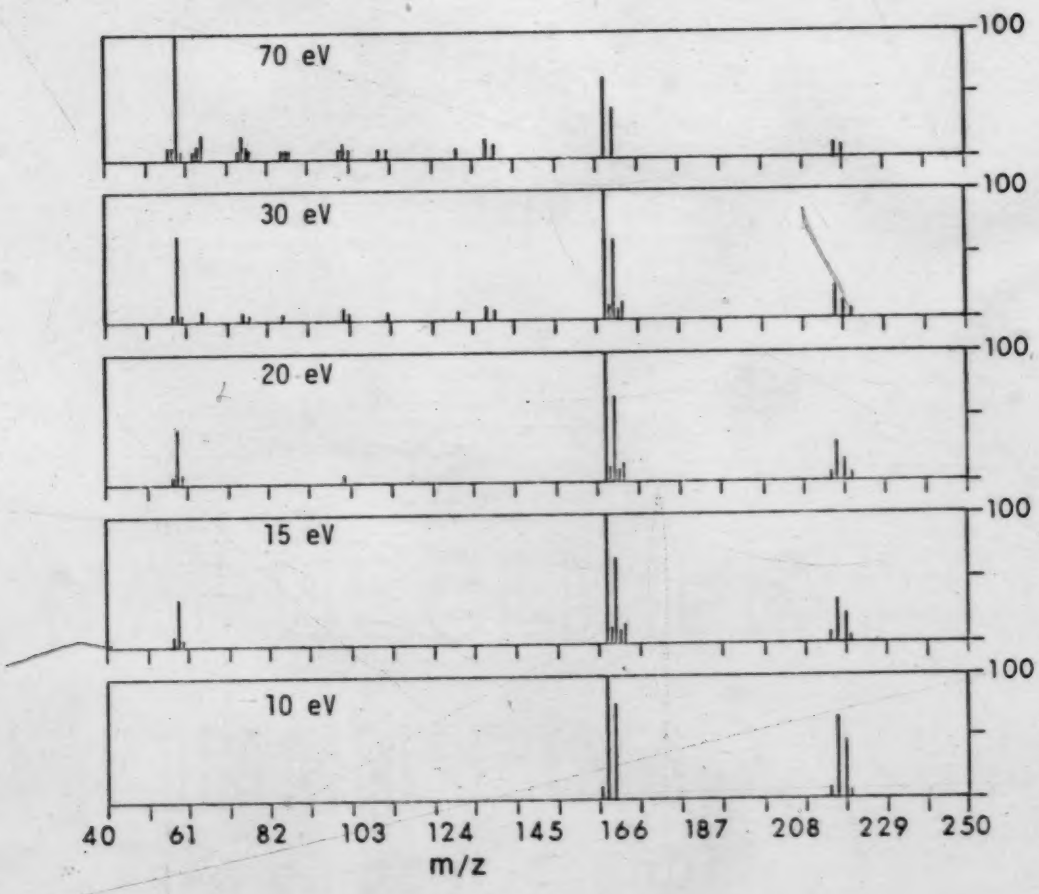


Figure 24. Effect of ion source voltage (10-70 eV) on the abundance of fragment ions in the electron impact mass spectrum of 2,4-dichlorophenyl propionate.

The propionate and acetate derivatives have different retention times under identical GLC conditions. The application of both SIM programs to the analysis of unknown samples can be used to provide complimentary results for the characterization of phenols whose identities are in question. SIM-MS can also be used as an alternative method for the analysis of biological or environmental samples containing interferences which complicate the detection of phenols by FID or ECD. Acyl derivatives of the phenols in this study could be readily detected at concentrations of 1 pmole/mL using SIM-MS.

Quantitative SIM analysis of phenols, using 4,6-DBC as an internal standard, was not possible. The linear relationship between concentration and ion peak area was inconsistent at low concentration levels. Generally, the fragmentation and chemical characteristics of a suitable reference compound for SIM quantitation must closely parallel those of the compound of interest. Bose and Fujiwara (189) prepared PCP benzoate derivatives and used the corresponding pentadeuterated benzoate of PCP as the internal standard for quantitative SIM analysis. Ingram et al. (175) described a mass spectrometric isotope dilution method using ^{18}O-labelled PCP as the reference compound. Wu et al. (178) used pentachlorophenetole as an internal standard for PCP analysis and a plot of peak height versus concentration was linear. For quantitative analysis, internal standards other than stable isotope-labelled analogues can be used but results are usually less accurate at low

concentrations due to differences in partition coefficient and chromatographic properties of the compound and internal standard. 4,6-DBC, therefore, could be used as a retention time marker, but was not suitable for quantitative analysis.

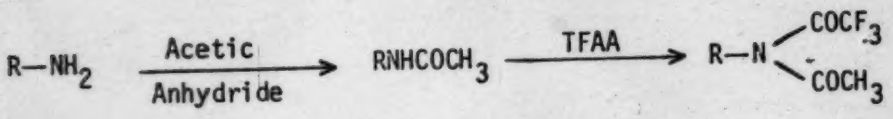
3. Derivatization and GLC Analysis of Aminophenols and Anilines

Present HPLC and GLC methods for the separation and quantitation of aminophenols, aniline and haloanilines in dilute aqueous solutions are inadequate for several reasons. The procedures are generally lengthy and satisfactory resolution of complex mixtures of aminophenol isomers, aniline and closely related haloanilines has not been achieved. The HPLC methods reported by Sternson and DeWitte (232, 233) permit the separation of 2- and 4-AP, but these studies did not include the 3-AP isomer. Lores et al. (39) were able to separate 3- and 4-CA using HPLC with solvent programming. Their study, however, did not include 3-Cl-4-MeA, which usually cannot be separated from 4-BrA. The HPLC method of Lores et al. was insensitive and required quantities greater than 10 ng on-column for UV detection. Solvent programming cannot be used with electrochemical detectors; increased sensitivity was achieved with the concomitant loss of compound resolution (39). Bradway and Shafik (137) evaluated the derivatization techniques currently available for the GLC analysis of a number of substituted anilines. All of the procedures required the extraction of the compounds of interest from water into an organic solvent prior to derivatization. Although numerous halogenated

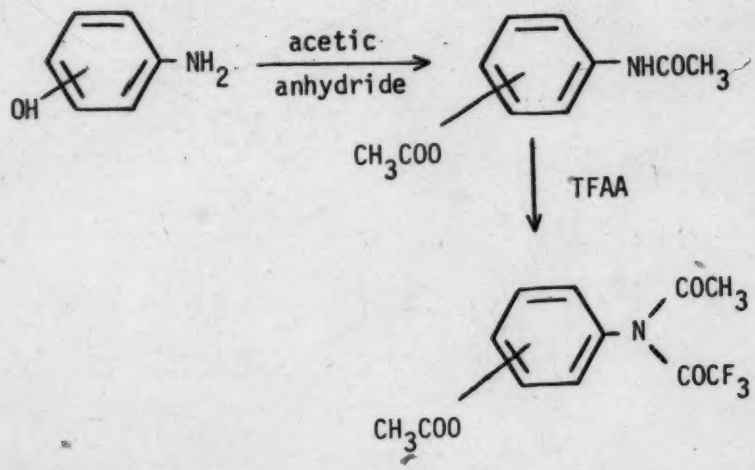
derivatives of anilines sensitive to ECD-GLC have been prepared, no similar derivatives of aminophenols have been reported.

In this study, a GLC method was developed which allows the efficient extraction of anilines and aminophenols from aqueous solution and which provides improved compound resolution and detection sensitivity. Acetate derivatives of ANI, 3-CA, 4-CA, 4-BrA, 3-Cl-4-MeA, 2-AP, 3-AP, 4-AP and internal standard BA were prepared directly in aqueous solution using acetic anhydride. The reaction produced N,O-diacetylated derivatives of the aminophenol isomers and N-acetylated derivatives of the anilines and BA. The acetates, efficiently extracted from the aqueous medium into methylene chloride, were then reacted with TFAA. Following aqueous acetylation, aminophenol and aniline derivatives had excellent GLC properties. Further reaction with TFAA produced highly electron capture sensitive derivatives, which reduced the minimal detection limit of the method. The reaction sequences are illustrated in Scheme 8. The reaction shown for ANI is typical of all the haloanilines included in this study. Following reaction with TFAA, the excess reagent must be removed prior to GLC analysis. The usual method of neutralizing TFAA with an alkaline buffer caused some breakdown of the triacylated aminophenols and diacylated BA and anilines. Excess TFAA could not be removed by evaporation to dryness since the derivatives were volatile and subject to uncontrolled losses. Cyclohexane (b.p., 81°C) was added, prior to concentration with a gentle stream of nitrogen, to avoid evaporative losses and to allow the removal of traces of TFAA (b.p., 40°C).

A. Derivatization of aniline ($R = C_6H_5$) and benzylamine ($R = C_6H_5CH_2$):



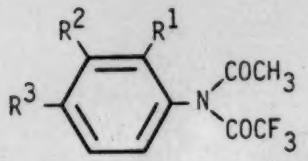
B. Derivatization of aminophenols:



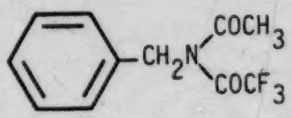
Scheme 8.

The structures of the trifluoroacetylated products depicted in Scheme 8 were confirmed using EI-MS and CI-MS. The major fragment ions in the EI mass spectrum of each derivative are identified in Table XVIII; plausible pathways that explain the formation of these

Table XVIII. Diagnostic Ions in the Spectra of the N-acetyl, N-trifluoroacetyl Derivatives of the Substituted Anilines (I) and Internal Standard, Benzylamine (II) and N,O-diacetyl, N-trifluoroacetyl Derivatives of Aminophenols



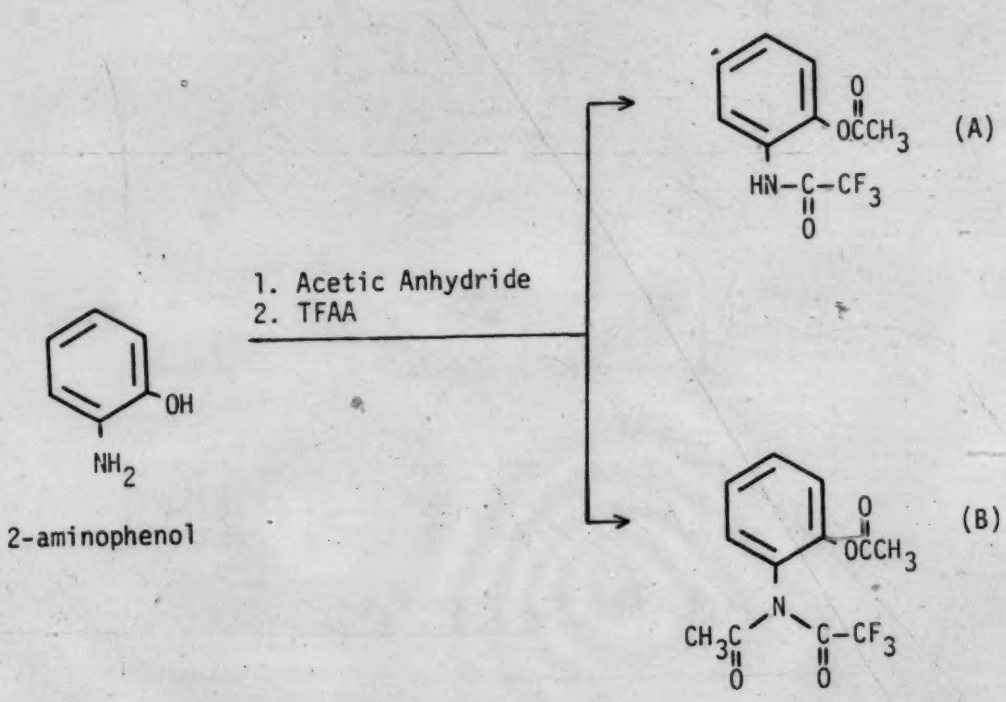
I



II

	Derivatives			m/z (% Relative Abundance)
	R^1	R^2	R^3	
I	-H	-H	-H	231 (absent) [M^+]; 190(9); 189(100); 120(42); 92(10); 69(10); 43(84)
I	-H	-Cl	-H	267(1.5); 265(4)[M^+]; 225(35); 223(100); 156(9); 154(30); 128(5); 126(2); 43(71)
I	-H	-H	-Cl	265(3)[M^+]; 225(33); 223(100); 156(8); 154(30); 128(9); 126(12); 43(76)
I	-H	-Cl	$-CH_3$	281(2); 279(6)[M^+]; 239(34); 237(100); 170(8); 168(28); 142(8); 140(12); 69(8); 43(60)
I	-H	-H	-Br	311(5); 309(6)[M^+]; 269(96); 267(100); 200(36); 198(34); 172(28); 170(20); 69(20); 43(95)
I	$-OCCH_3$ (O)	-H	-H	289[M^+](absent); 247(27); 205(76); 187(45); 136(100); 108(23); 69(10); 43(67)
I	-H	$-OCCH_3$ (O)	-H	289[M^+](2); 247(34); 205(100); 149(32); 136(26); 43(3)
I	-H	-H	$-OCCH_3$ (O)	289[M^+](3); 247(29); 206(10); 205(100); 108(6); 69(2); 43(3)
II				245(5)[M^+]; 203(100); 202(75); 106(37); 91(28); 69(15); 43(91)

ions are indicated in Figure 25 and Figure 26. As shown in Table XVIII, the m/z 289 molecular ion of the 2-AP derivative was absent from the EI mass spectrum. The exact structure of this derivative was, therefore, in some doubt. As a result of steric hindrance, the diacylated derivative (A) shown in Scheme 9 could have been formed rather than the triacylated derivative (B).



Scheme 9.

CI-MS was used to unequivocally characterize the nature of the 2-AP derivative; the spectrum obtained is shown in Figure 27. The presence of the quasimolecular ion (MH^+) at m/z 290 confirmed that 2-AP also formed the triacylated derivative (B).

General fragmentation pathway:

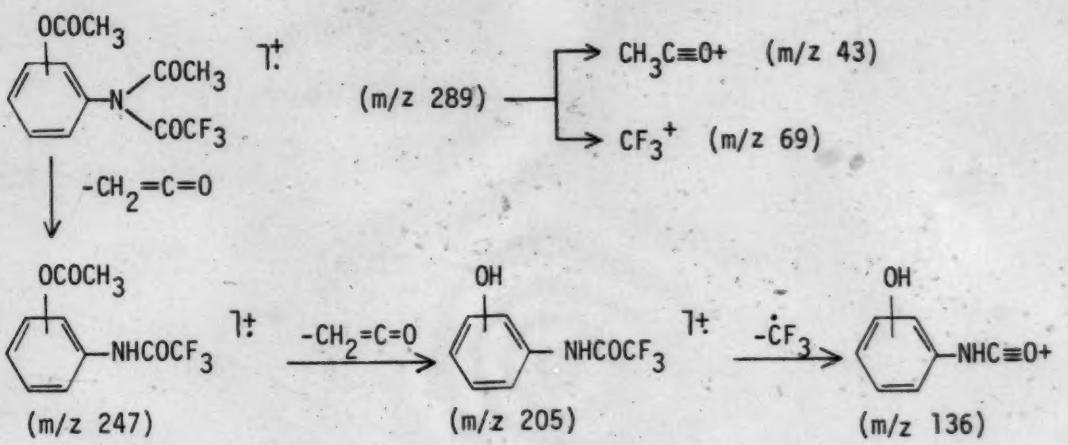
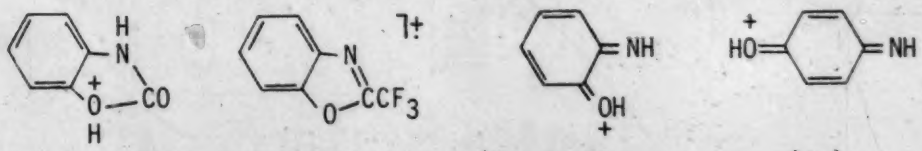


Figure 25. Mass spectral fragmentations of derivatized aminophenols.[1-4]

1. Molecular ion (m/z 289) is of low abundance in the electron impact mass spectra of the m- and p-isomers, and absent from the spectrum of the o-isomer. Using chemical ionization mass spectrometry the molecular weight of o-, m-and p-aminophenol derivatives was confirmed to be 289.

2. In the spectrum of the o-isomer, the ion m/z 136 was the base peak. This may be due to the formation of a cyclized fragment ion (i).



3. Only the spectrum of the o-isomer contains an abundant ion, m/z 187. This is formed by the expulsion of CH_3COOH from ion, m/z 247. Such an 'ortho effect' would yield ion (ii).

4. The spectra of the o- and p-isomers contain weak ions, m/z 108 which are presumed to possess the structures (iii) and (iv) respectively, and are formed by the expulsion of CO from ion, m/z 136. A quinonoid ion cannot be formed from the m-isomer.

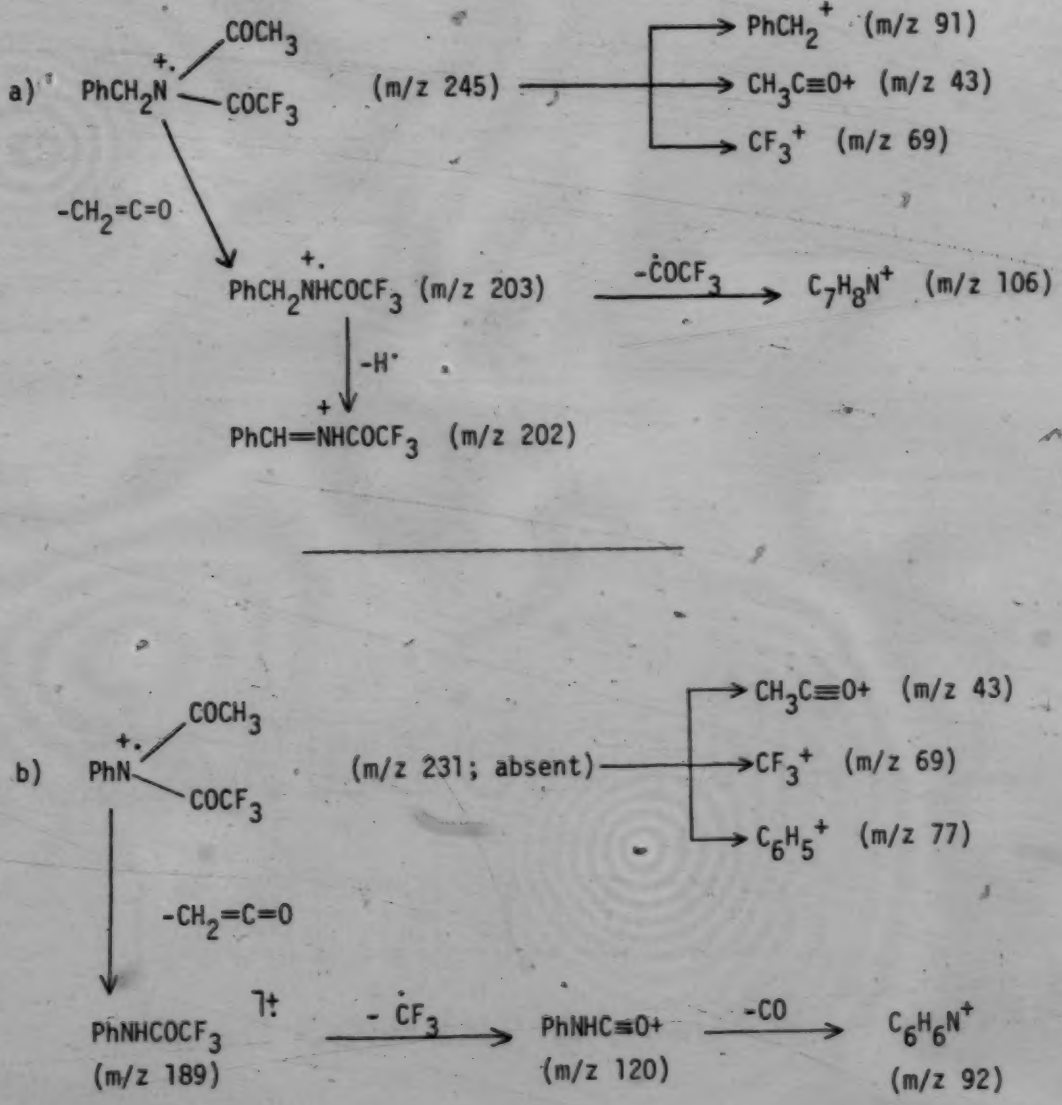


Figure 26. Mass spectral fragmentation of a) derivatized benzylamine and b) derivatized aniline.

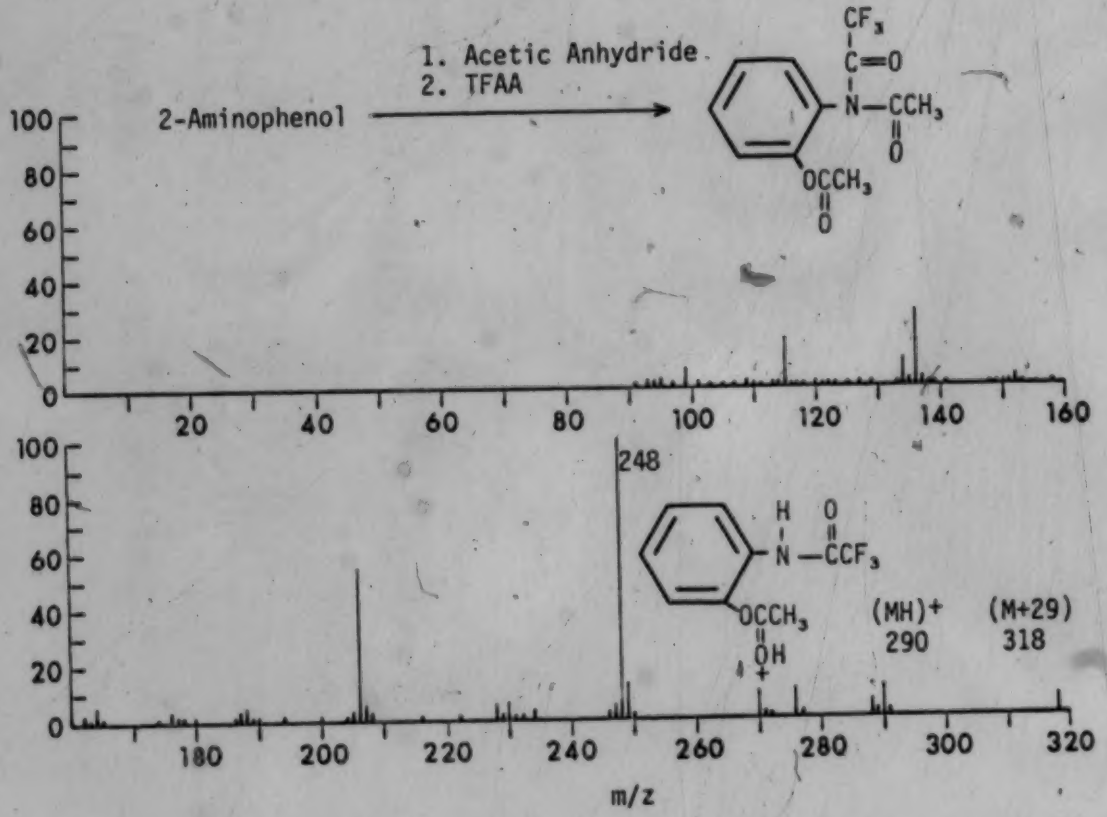


Figure 27. Chemical ionization mass spectrum of the derivative of 2-aminophenol obtained following aqueous acetylation, extraction and further reaction with trifluoroacetic anhydride.

2-AP, 3-AP, 4-AP and ANI were added, at concentrations of 5-500 nmoles each, to distilled water samples (100 mL) containing 50 nmoles of BA. The prepared standard samples were acetylated, extracted and trifluoroacetylated according to the described two-step procedure. Linear calibration graphs were obtained by FID-GLC using a packed 3.0% OV-17 column. Figure 28 is the gas chromatogram of one of the extracts (50 nmoles each of BA, ANI, 2-, 3- and 4-AP/100 mL) prepared for the calibration graph. Both the acetic anhydride and TFAA reactions were quantitative; peaks corresponding to the underivatized aminophenols or acetates were absent from the trace. The minimum detectable concentration of each of the derivatized compounds was 5 nmoles/100 mL. Since only 1 μL of the final preparation (approximately 20 μL) was chromatographed, this represents an injection of 0.25 nmoles "on-column".

The use of a capillary column improved the resolution of the aminophenol derivatives (Figure 29) and the incorporation of an ECD greatly enhanced the sensitivity of the procedure. Calibration graphs obtained using this system were linear over the concentration range of 0.25-500 nmoles for all four compounds when dissolved in 100 mL of distilled water. A segment of these graphs is shown in Figure 30. The minimum detectable concentration was 0.1 nmole/100 mL for each derivatized amine (equivalent to 0.33 pmoles "on-column").

As shown in Table XI, the U.S.A. has not set effluent limits for the aminophenols and anilines. The U.S.S.R. (1, 52) has set toxicological limits of approximately 100 nmoles/100 mL for ANI, 10 nmoles/100 mL for 2-AP and 50 nmoles/100 mL for 3- and 4-AP. The

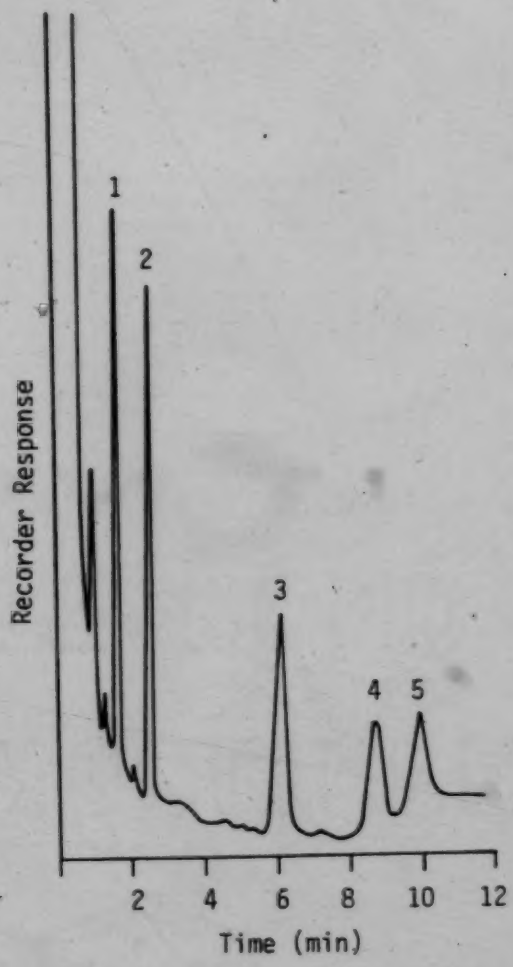


Figure 28. Gas-liquid chromatographic separation of derivatives of aniline and the aminophenols using a packed column and flame ionization detection. Peak 1: aniline; 2: benzylamine; 3: 2-aminophenol; 4: 3-aminophenol; 5: 4-aminophenol. A 100 mL distilled water sample was spiked with 50 nmoles of each compound. Chromatographic conditions: 3% OV-17, isothermal at 155°C (1.68m, 80-100 Chromosorb W).

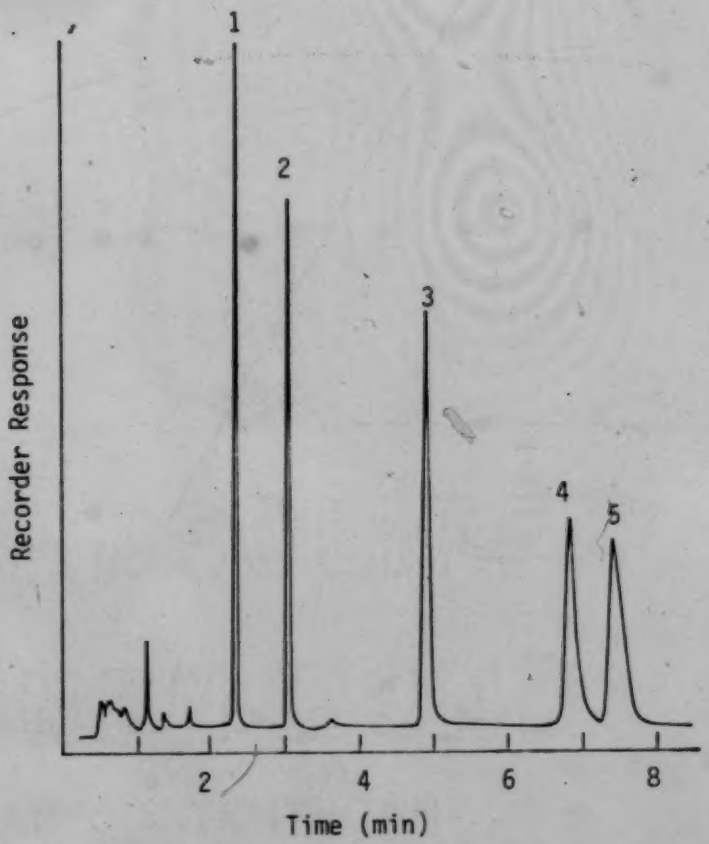


Figure 29. Gas-liquid chromatographic separation of derivatives of aniline and the aminophenol isomers using an SP-2100 capillary column with electron capture detection. Peak 1: aniline; 2: benzylamine; 3: 2-aminophenol; 4: 3-aminophenol; 5: 4-aminophenol. Chromatographic conditions are described in the text.

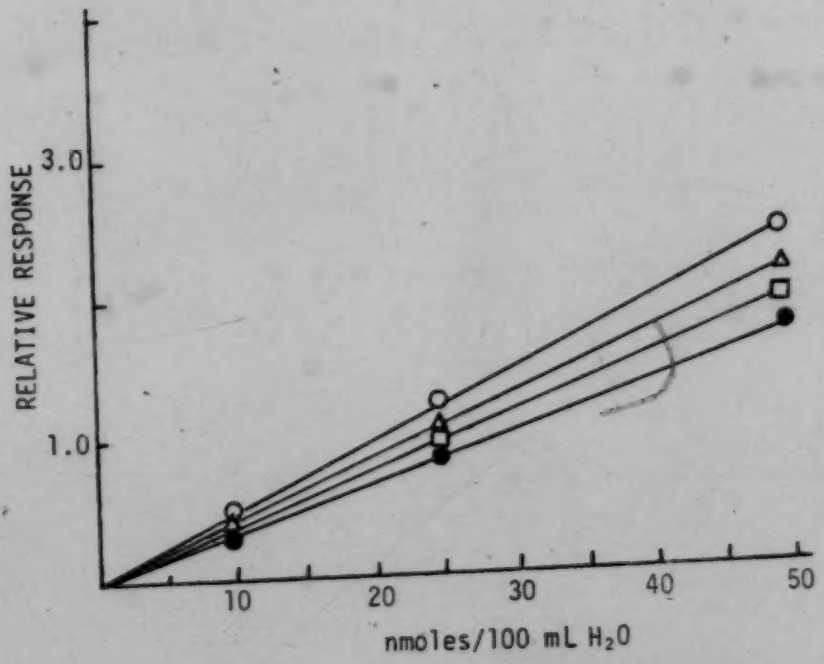


Figure 30. Calibration graphs for acyl derivatives of aminophenol isomers and aniline in the concentration range 5-50 nmoles in 100 mL distilled water. ○: 2-aminophenol; △: 3-aminophenol; □: aniline; ●: 4-aminophenol. Chromatographic conditions for the SP-2100 capillary column equipped with an ECD are given in the text. Benzylamine (2.5 nmoles/100 mL) was used as the internal standard for calibration.

procedure developed here, therefore, is capable of measuring these compounds at concentrations well below these acceptable limits. A number of procedures have been reported for the analysis of aminophenols and aniline. It is difficult to compare the sensitivity achieved using the developed aqueous acetylation-trifluoroacetylation method to those in the literature because units expressing the "minimum detectable" concentration are often inconsistent and vague. Various investigators report detection sensitivities in μg per volume (236), moles per liter (232, 233) or ppm (137), but fail to specify the actual volume analyzed and do not indicate the minimum volume which could be injected onto the HPLC or GLC column. Kulikova et al. (299) were able to detect as little as 150 ng of ANI when a trifluoromethoxytetrafluoropropionyl anilide was prepared following benzene extraction of a 50 mL water sample. In contrast the two-step acylation method developed in the present study permits the analysis of aqueous solutions containing 10 ng/100 mL of ANI and APs using capillary ECD-GLC. The sensitivity of the capillary ECD-GLC procedure also compares favourably with existing HPLC methods. With amperometric monitoring of the HPLC column effluent, Sternson and DeWitte (233) reported a detection limit of 1×10^{-8} M, 5×10^{-8} M and 1×10^{-6} M for 4-AP, 2-AP and ANI, respectively. 3-AP was not included and their study did not specify the actual water volume extracted. Using the two-step procedure developed in this study, the capillary ECD-GLC detection limit for acylated ANI, 2-, 3- and 4-AP is equivalent to 1×10^{-9} M. Furthermore, it was possible to detect 0.1 nmoles of all four compounds in a 100 mL aliquot of water;

analysis of a one litre sample was not required. The minimum detectable concentrations of ANI, 2-, 3- and 4-AP, therefore, have been reduced significantly by the developed procedure.

Many electron-capture sensitive derivatives of ANI, 3-CA, 4-CA, 3-Cl-4-MeA and 4-BrA, prepared to improve the GLC detection sensitivity, do not allow simultaneous resolution of the halogenated anilines. Of the various derivatives prepared by Bradway and Shafik (137), only those obtained by reaction with PFPA and HFBA gave clean chromatograms at low concentrations. PFPA was considered the reagent of choice, even though derivatives of 3-CA and 4-BrA could not be resolved from 4-CA and 3-Cl-4-MeA, respectively.

In the present study, following the aqueous acetylation / extraction procedure N-acetates of 3-Cl-4-MeA could be easily separated from 4-BrA (Figure 31) using a 0.2% Carbowax 20M column. N-Acetyl-3-chloroaniline and N-acetyl-4-chloroaniline still had virtually identical retention times and could not be resolved.

The acetylated aniline derivatives were further reacted with TFAA. As shown in Figure 32, complete resolution of the N-acetyl-N-trifluoroacetyl anilines and BA was achieved within 7 min using a 3% OV-17 column (1.68 m) run under isothermal conditions at 140°C. While many of the herbicides listed in Table I are degraded to form 3- and 4-CA, herbicides degrading to 2-CA are not commonly available. The 2-CA isomer has not been included in GLC or HPLC methods reported in the literature for the analysis of halosubstituted anilines. As shown in Figure 33, the N-acetyl-N-trifluoroacetyl derivatives of 2- and 3-CA could not be completely resolved.

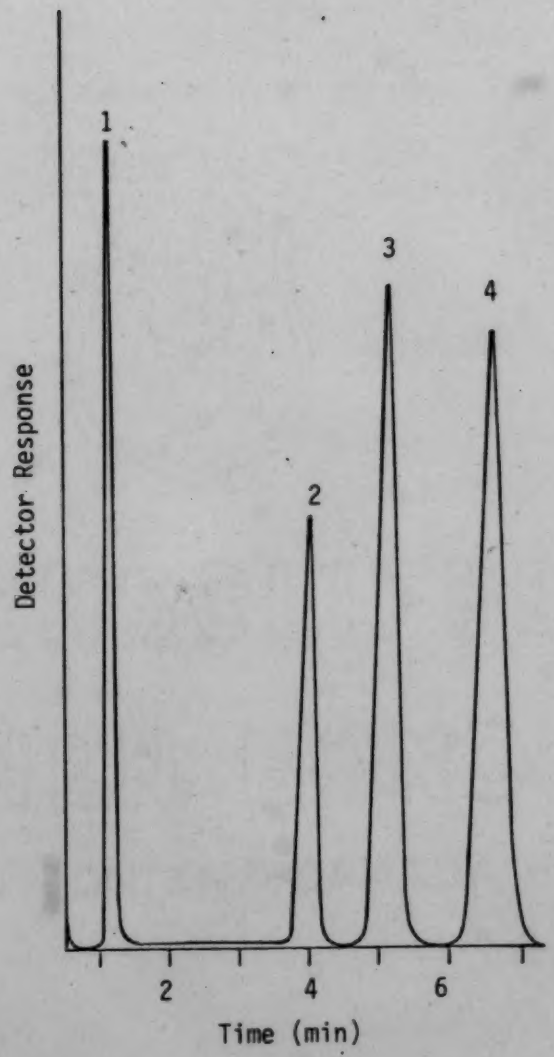


Figure 31. Computer-reconstructed total ion trace of N-acetylated anilines using a 0.2% Carbowax column. Peak identification of acetylated derivatives 1: benzylamine; 2: 3-chloroaniline and 4-chloroaniline; 3: 3-chloro-4-methylaniline; 4: 4-bromoaniline. Chromatographic conditions: Isothermal at 190°C.

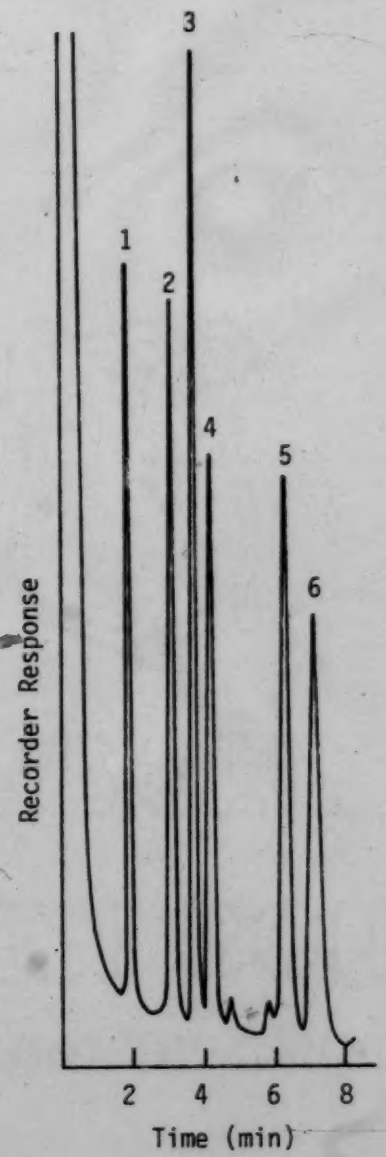


Figure 32. Gas-liquid chromatographic separation of N-acetyl-N-trifluoroacetylanilines using a 3% OV-17 column (1.68 m). Peaks are acyl derivatives of 1: aniline; 2: benzylamine; 3: 3-chloroaniline; 4: 4-chloroaniline; 5: 3-chloro-4-methylaniline; 6: 4-bromoaniline. Fifty nmoles of each compound in 100 mL H_2O were acetylated and trifluoroacetylated as described in the text. Chromatographic conditions: Isothermal at 140°C.

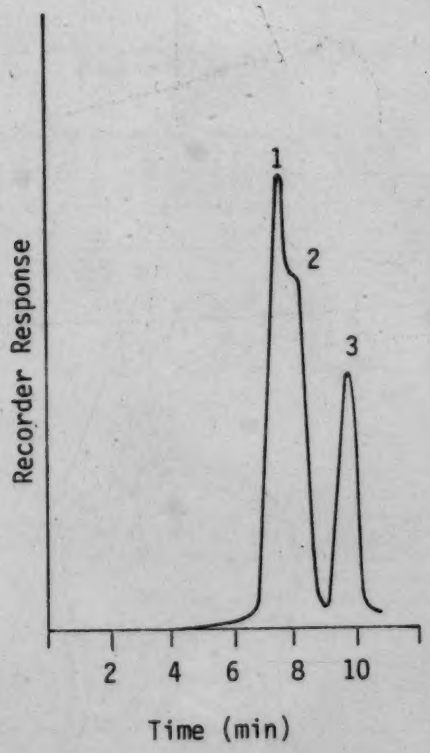


Figure 33. Gas-liquid chromatographic separation of 2-chloroaniline (1), 3-chloroaniline (2) and 4-chloroaniline (3) as N-acetyl-N-trifluoroacetyl derivatives. Chromatographic conditions: 3% OV-17 (1.68 m), 130°C isothermal.

The trifluoroacetylation reaction proceeded to greater than 97% completion for all of the anilines. Traces of residual acetanilides, which were present following trifluoroacetylation, were much less volatile and did not interfere with the GLC analysis of the N-acetyl-N-trifluoroacetyl derivatives. The diacylated anilines were stable for 2-3 hr; however, only 20-50% of the diacyl compounds remained after storage at 4 °C for 14 hr. N-Acetanilides and N-trifluoroacetanilides were present as decomposition products (Figure 34).

Calibration graphs for each of the derivatized anilines were linear over the 5-100 nmoles/100 mL concentration range using the 3% OV-17 column (1.68 m) and FID. The minimum detectable concentration was 1 nmole/100 mL. Since only 1 µL of the final derivative preparation (20 µL) was chromatographed, this represents an injection of 0.05 nmoles "on-column". The use of an ECD in combination with a capillary column substantially increased the detection sensitivity. The calibration graphs obtained (Figure 35) were linear over the concentration range 1-20 nmoles for each of the five anilines when dissolved in 100 mL of distilled water. The minimum detectable concentrations for aniline and each of the substituted anilines were 0.1 nmoles/100 mL and 0.05 nmoles/100 mL, respectively. This is a substantial improvement over the ECD-GLC limit of between 3.2-6.3 nmoles/100 mL reported (300) for 3,4-dichloroaniline following derivatization with CAA. The ECD detection limit determined in the present study represents an "on-column" injection of 0.33 pmoles of ANI and 0.17 pmoles of the substituted anilines. The two-step acetylation/trifluoroacetylation procedure for GLC not only provides

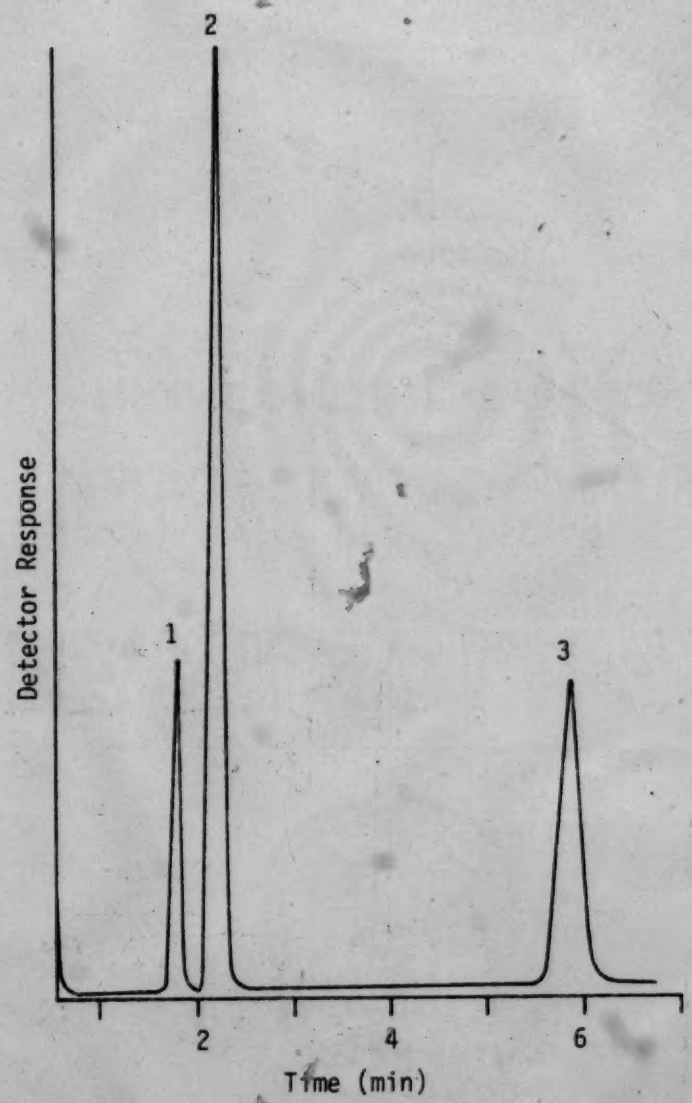


Figure 34. Computer-reconstructed total ion trace of decomposition products of N-acetyl-N-trifluoroacetyl-3-chloroaniline following 14 hr storage at 4°C. Peak identification 1: N-trifluoroacetyl-3-chloroaniline; 2: N-acetyl-N-trifluoroacetyl-3-chloroaniline; 3: N-acetyl-3-chloroaniline. Chromatographic conditions: 3% OV-17 (1.68 m), 170-200°C at 8°/min.

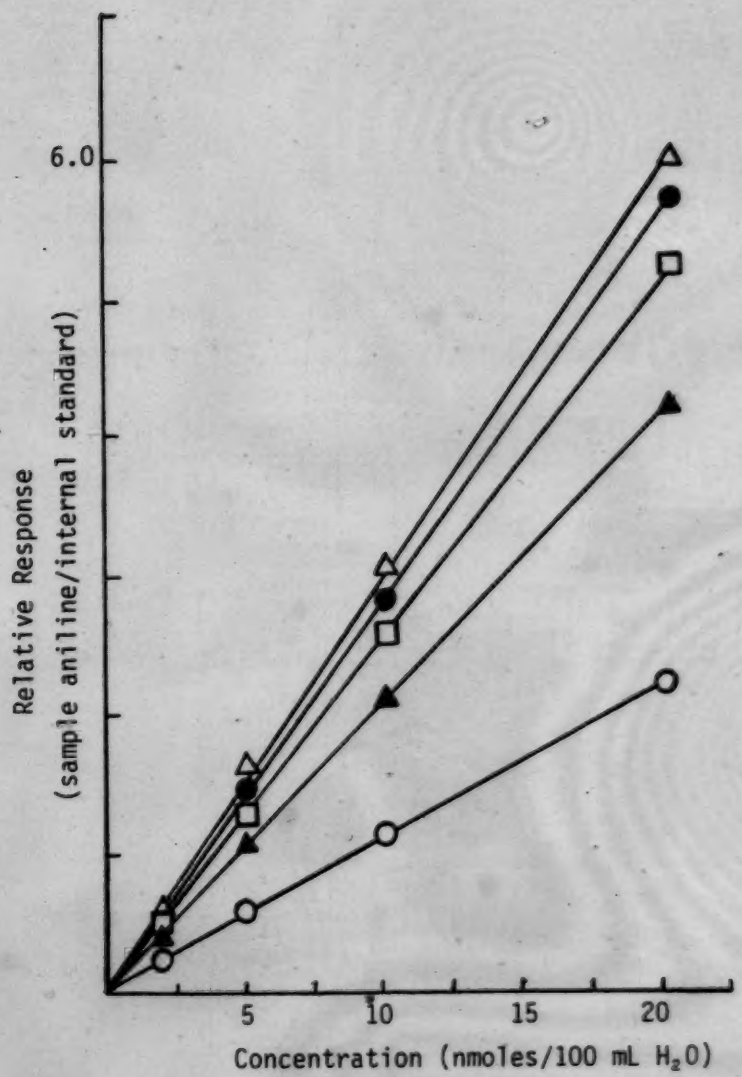


Figure 35. ECD calibration graphs for diacylated aniline derivatives in the concentration range 1-20 nmoles in 100 mL distilled water.

○, Aniline; ▲, 3-chloroaniline; ●, 4-chloroaniline; □, 3-chloro-4-methylaniline; △, 4-bromoaniline.

Benzylamine (5 nmoles/100 mL) was used as the internal standard for calibration.

improved sensitivity for anilines and aminophenols, but also allows the analysis of complex aniline mixtures which were unresolved when other analytical procedures were utilized.

Singh et al. (191) have reported the derivatization of ethylene thiourea in water using DCAA. In this one-step extraction and derivatization method, ethylene thiourea was partitioned from water into the organic phase which contained DCAA using acetonitrile as a phase transfer reagent. For comparison with the aqueous acetylation procedure developed in this study, the phase transfer method developed by Singh et al. was applied to the derivatization of the aminophenols and anilines with CAA and DCAA. If these chloroacylation reactions were successful, an electron-capture sensitive derivative would be formed in the direct aqueous derivatization step. The detection limit of these derivatives could be further enhanced by reaction with TFAA. The phase transfer reactions of CAA with aminophenols, however, produced both monoacylated and diacylated products in almost equal concentrations. As shown in Figure 36, the two products of the CAA phase transfer reaction with 4-AP were identified by their mass spectra. The phase transfer reactions of DCAA with anilines also did not proceed to completion. When 4-CA was reacted with DCAA followed by TFAA, the complex chromatogram shown in Figure 37 was obtained. The mass spectra of the three reaction products identified as trifluoroacetyl-(A), dichloroacetyl-(B) and trifluoroacetyl dichloroacetyl-4-chloroaniline (C), are shown in Figure 38. The presence of the large dichloroacetyl peak may indicate that the trifluoroacetyl dichloroacetyl derivative was unstable and subject to decomposition.

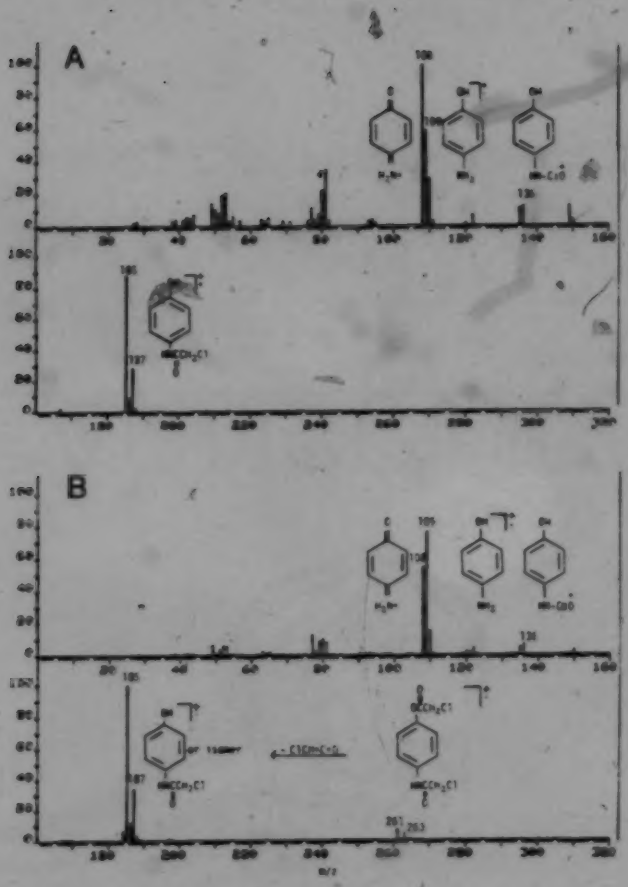


Figure 36. The monoacyl (A) and diacyl (B) derivatives of 4-aminophenol, produced by phase transfer reaction with chloroacetic anhydride, are easily identified by their mass spectra.

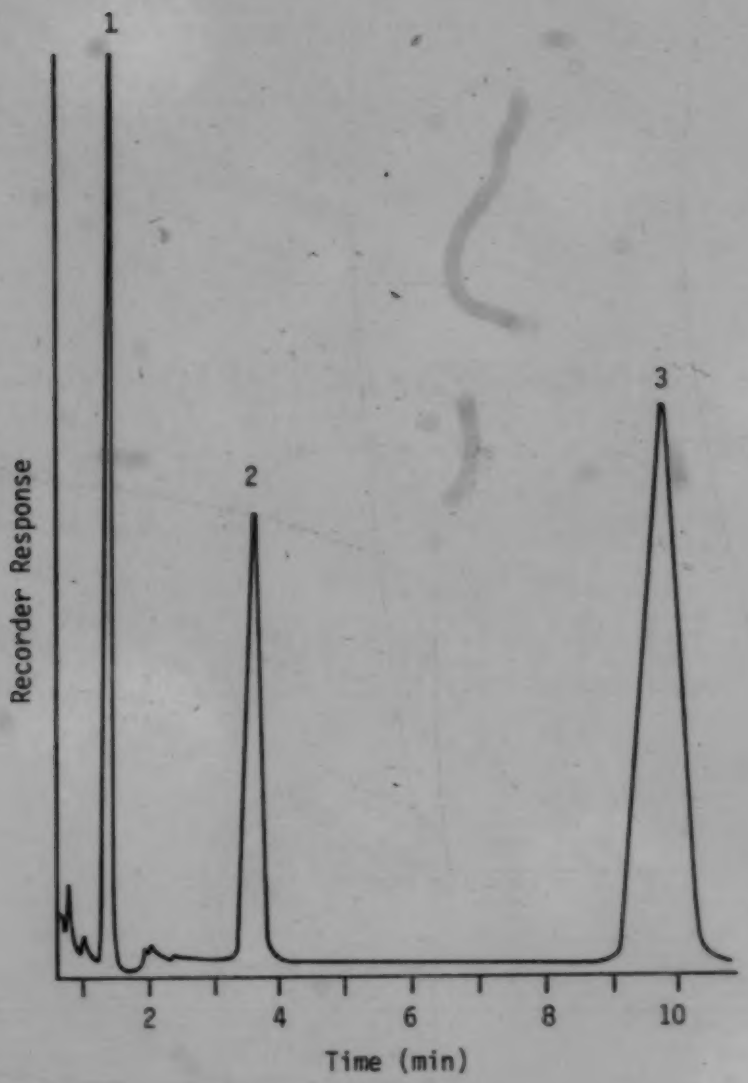


Figure 37. Computer-reconstructed total ion trace of the products obtained following the derivatization of 4-chloroaniline with DCAA and TFAA in a two-step procedure. Peak 1: N-trifluoroacetyl-4-chloroaniline; 2: N-dichloroacetyl-N-trifluoroacetyl-4-chloroaniline; 3: N-dichloroacetyl-4-chloroaniline. Chromatographic conditions: 3% OV-17, isothermal at 190°C.

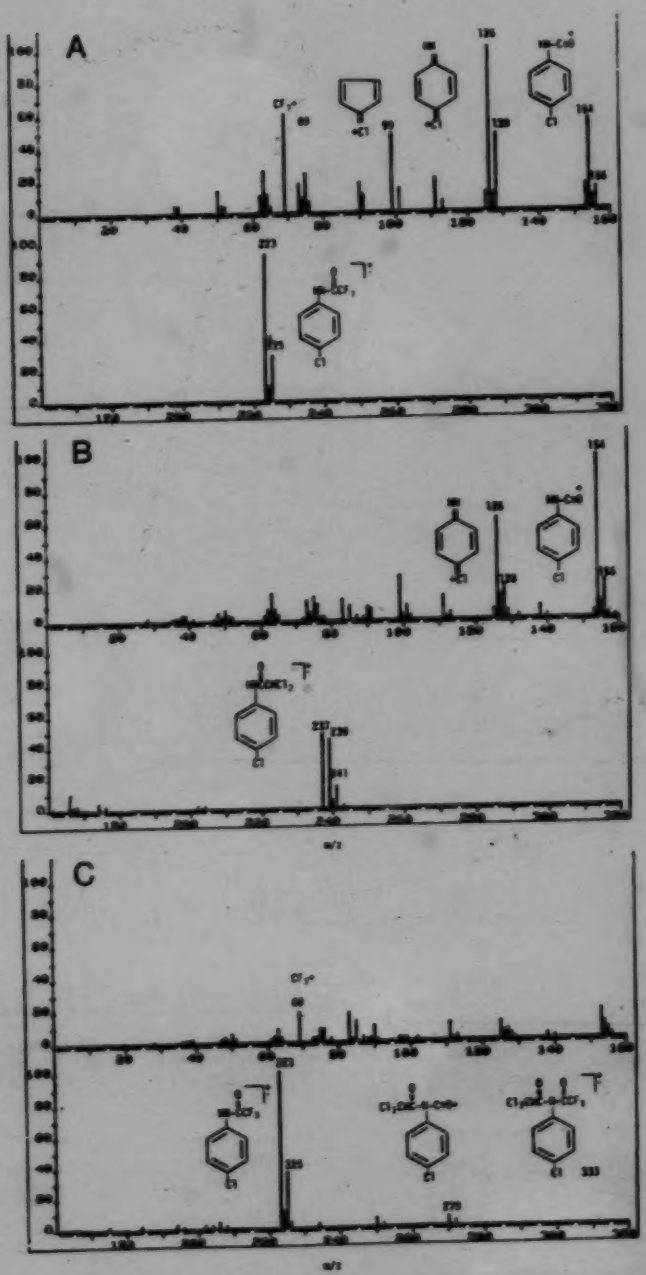


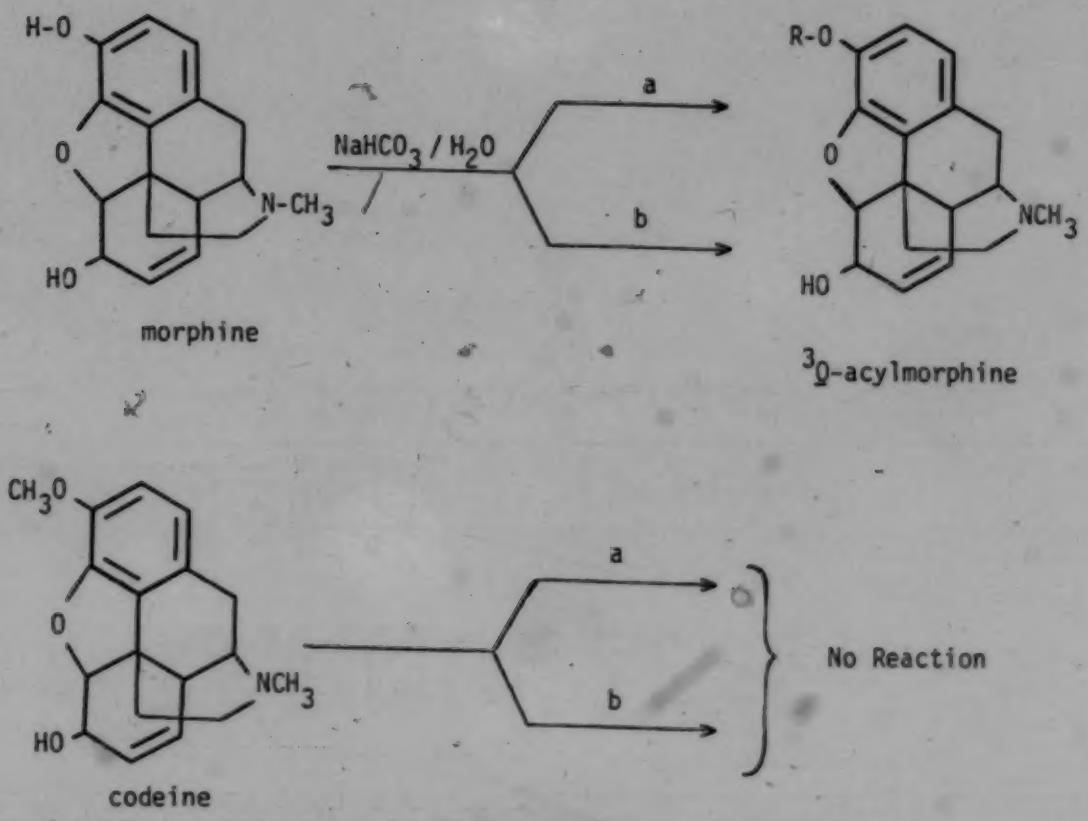
Figure 38. Three reaction products, identified by their mass spectra as trifluoroacetyl-(A), dichloroacetyl-(B) and trifluoroacetyl dichloroacetyl-4-chloroaniline (C), were obtained by reaction of 4-chloroaniline with dichloroacetic anhydride followed by trifluoroacetic anhydride.

4. Derivatization and GLC Analysis of Morphine Alkaloids

In any quantitative GLC method for the analysis of morphine, the most critical step is sample extraction. Morphine is an amphoteric molecule and its quantitative recovery from aqueous solution is difficult; long and tedious extraction sequences are common features of most GLC methods. Cole *et al.* (268), however, described an extractive alkylation method which dramatically simplified the recovery and derivatization of morphine. In the work reported here, the use of aqueous acylation for the simultaneous extraction and derivatization of morphine was investigated as a simple alternative to the published extractive alkylation method.

In alkaline ($NaHCO_3$) aqueous solution, morphine reacts quantitatively with both propionic and acetic anhydride to produce the corresponding ^{3}*O*-acylmorphine derivatives (Scheme 10). While diacetyl derivatives are obtained following the reaction of morphine with acetic anhydride in pyridine (280), the alcoholic hydroxyl group is not acylated in the presence of water. As expected, the reaction of codeine with acyl anhydride reagents in alkaline aqueous medium (Scheme 10) failed to produce acylated derivatives.

Plausible fragmentation sequences which explain the formation of the major ions in the mass spectrum of ^{3}*O*-acetylmorphine (Figure 39) are indicated in Figure 40. Following the loss of ketene from the molecular ion, two possible fragmentation pathways of the odd electron ion m/z 285 (Ia and Ib) can be suggested to explain the other major ions in the spectrum (301-304). The mass spectrum of



a) $CH_3\overset{O}{\overset{\|}{C}}O\overset{O}{\overset{\|}{C}}CH_3$, R = $CH_3\overset{O}{\overset{\|}{C}}-$

b) $CH_3CH_2\overset{O}{\overset{\|}{C}}O\overset{O}{\overset{\|}{C}}CH_2CH_3$, R = $CH_3CH_2\overset{O}{\overset{\|}{C}}-$

Scheme 10.

^{3}O-propionylmorphine could also be described by fragmentation sequences similar to those shown in Figure 40.

The ^{3}O-acylmorphine derivatives were quantitatively extracted from aqueous solution into chloroform:isopropanol. Unlike the commonly prepared perfluoroacyl and silyl derivatives, the ^{3}O-acylmorphine compounds are not subject to decomposition in the presence of moisture. The calibration graph for ^{3}O-propionylmorphine,

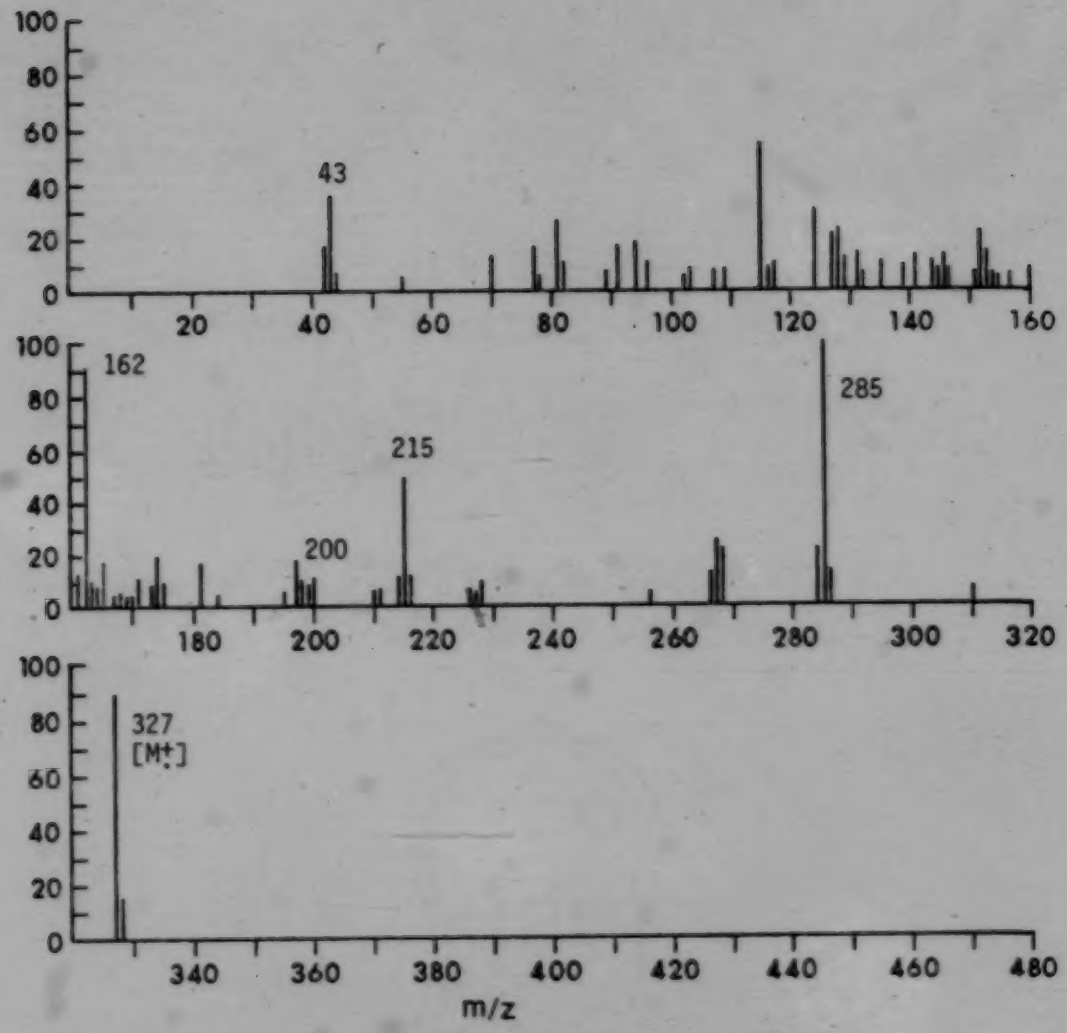


Figure 39. As indicated by the mass spectrum, the ^{3}O-acetylmorphine derivative is produced by the reaction of morphine with acetic anhydride in aqueous alkaline solution.

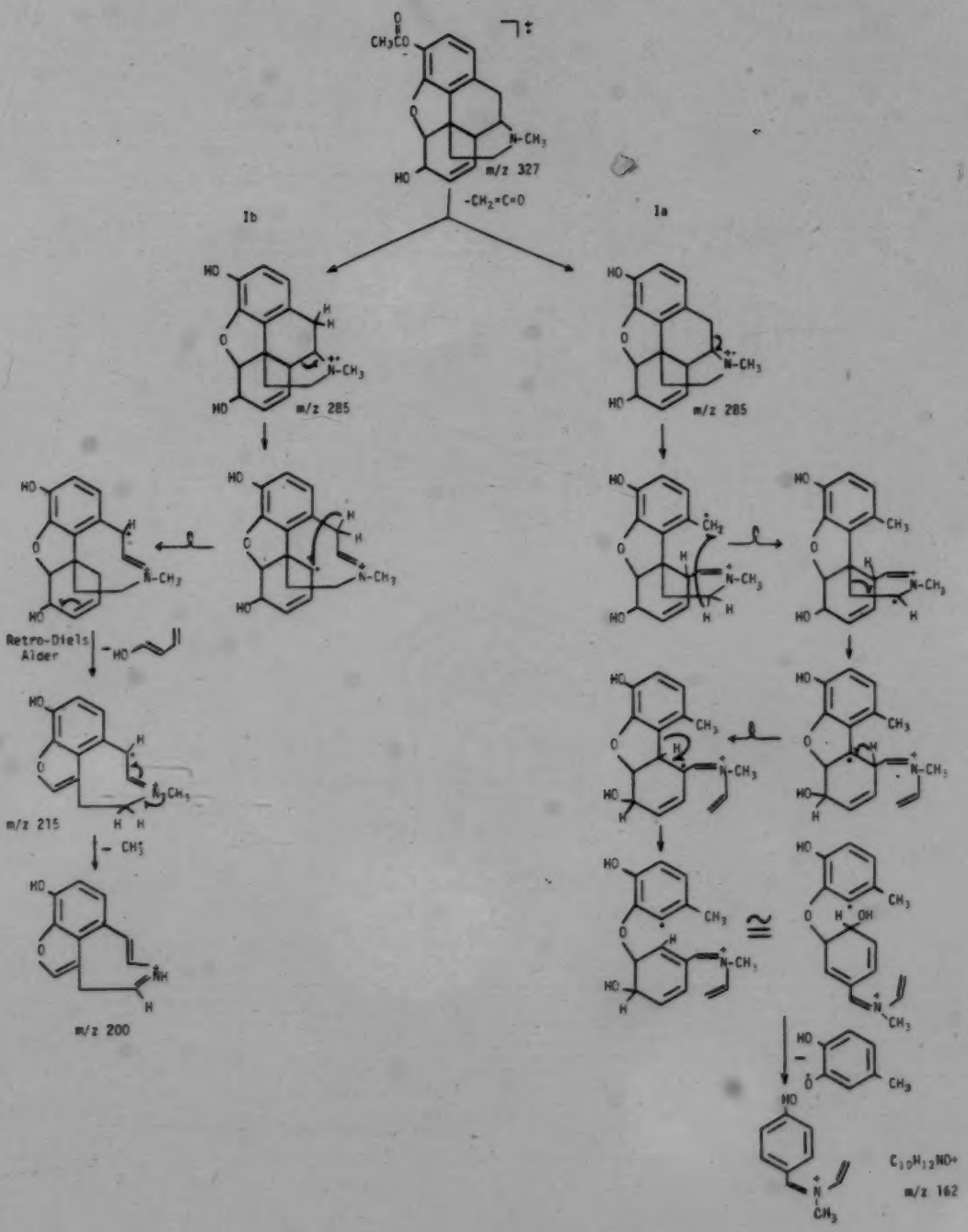
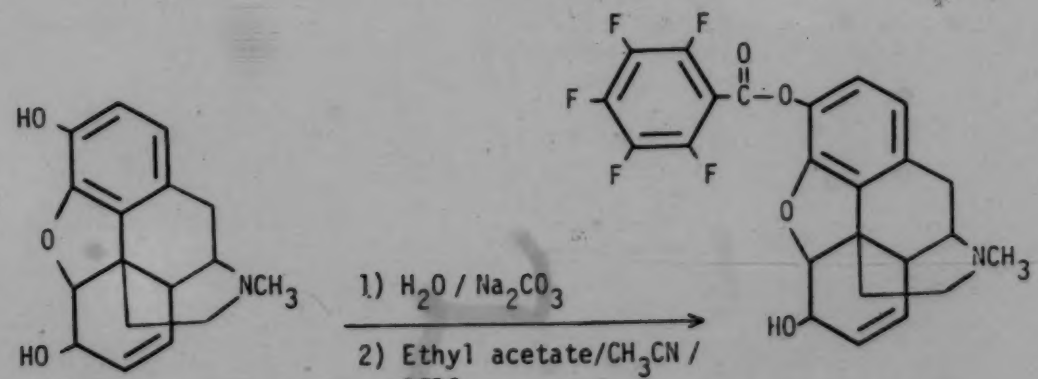


Figure 40. Plausible mass spectral fragmentation pathways of [3]Q-acetylmorphine.

prepared over a concentration range of 0.1-1.0 mg/mL, was linear; the FID-GLC detection limit was 200 ng/mL. ^{3}O-acylmorphine derivatives were further reacted with TFAA, HFBA or PFPA in order to prepare compounds suitable for analysis by the more sensitive electron capture detector. Following the perfluoroacylation step, however, complex chromatograms containing mixtures of monoacylated and diacylated morphine derivatives were obtained.

A morphine derivative amenable to ECD-GLC could more easily be prepared by phase transfer reaction with PFBC (194). PFBC, like acetic and propionic anhydride, reacted only with the phenolic hydroxyl group in aqueous solution to produce ^{3}O-pentafluorobenzoyl-morphine as shown in Scheme 11.



morphine

^{3}O-pentafluoro-benzoylmorphine

Scheme 11.

^{3}O-Pentafluorobenzoylmorphine is substantially less volatile than propionyl or acetyl derivatives. Using a 3% OV-17 (0.84 m) column, high temperatures (280 °C) were required for GLC analysis and detection sensitivity was limited by the broad peaks obtained at low concentrations. The ECD-GLC detection limit for the pentafluorobenzoyl derivative was 10 ng/mL. Dahlstrom et al. (273) reported a GLC method with an electron capture detection sensitivity of 0.75 ng for morphine. The procedure, however, involved a complex extraction of morphine from the plasma sample prior to derivatization with PFPA. Though poorer detection sensitivities were achieved using acetic anhydride, propionic anhydride or PFBC, the aqueous acylation and phase transfer procedures were rapid and simple. Reasonably sensitive analysis was still possible and quantitative extraction was attained in a single step.

B. METHOD APPLICATION - ANALYSIS OF BIOLOGICAL, ENVIRONMENTAL AND FORENSIC SAMPLES

In parallel with all of the biological, environmental and forensic samples analyzed, a distilled water sample of comparable volume was run through all acylation, cleanup and concentration steps. Each "blank" ensured that no significant contamination was introduced during the analytical procedures. Calibration graphs for quantitative analysis were prepared using internal standard addition. Volumes of distilled water, comparable in size to the samples in each study, were spiked with appropriate concentrations of the authentic

standard stock solutions. Compounds were tentatively identified by comparing GLC retention time values against those obtained for standards similarly derivatized. Confirmation of identity was achieved by comparing the mass spectra of unknown compounds with those of authentic standards.

1. Urine Samples

a) Analysis of Anilines

Sensitive and specific methods are required to monitor environmental and occupational exposure to the many pesticides (Table I) which produce aniline and substituted-aniline urinary metabolites. It has been demonstrated that the aqueous acetylation-trifluoroacetylation method allows the resolution and sensitive detection of ANI, 3-CA, 4-CA, 4-BrA and 3-Cl-4-MeA. Application of the described analytical procedure to a 5 mL urine sample, to which 100 nmoles of BA and 50 nmoles of each aniline were added prior to acid hydrolysis, gave satisfactory results (Figure 41 A) using FID-GLC. The derivatized extract of a normal hydrolyzed urine sample (Figure 41 B) did not contain detectable herbicide metabolites nor large peaks which would interfere with the quantitation of 3-CA, 4-CA, 4-BrA or 3-Cl-4-MeA. At very low concentrations requiring high GLC detector gain, the aniline peak may be located in the solvent tail of some samples.

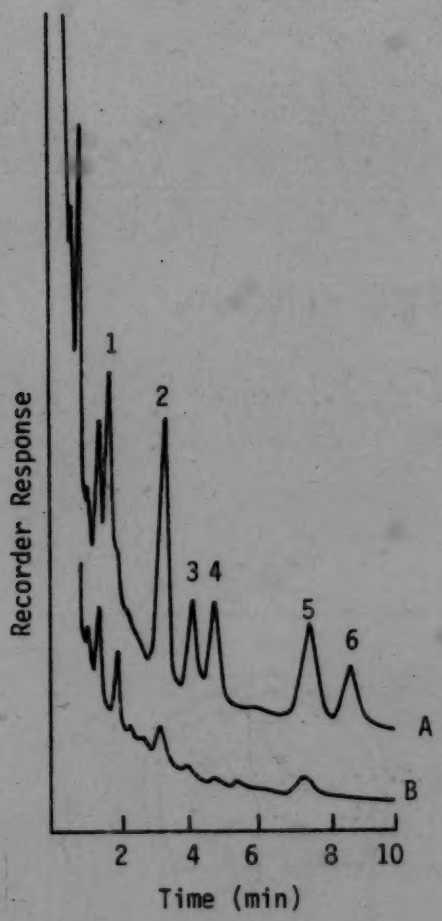
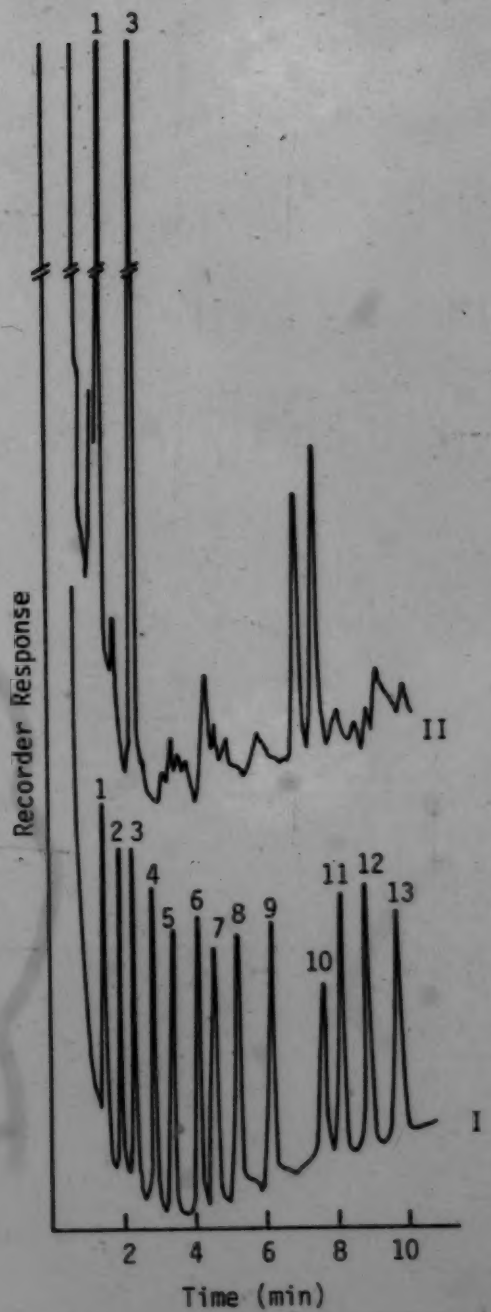


Figure 41. GLC analysis of derivatized extracts of hydrolyzed urine samples (5 mL) using a 3% OV-17 column with FID detection. A. Urine sample spiked with 50 nmoles of each aniline and 100 nmoles of benzylamine. B. Blank urine sample. Peaks are derivatives of 1: aniline; 2: benzylamine; 3: 3-chloroaniline; 4: 4-chloroaniline; 5: 3-chloro-4-methylaniline; 6: 4-bromoaniline. Chromatographic conditions: Isothermal at 140°C (1.68m, 80-100 mesh).

b) Analysis of Chlorophenols by Selected Ion Monitoring-Mass Spectrometry

The SIM programs shown in Tables XVI and XVII, designed to detect acetate and propionate derivatives of PHE, o-CRE, p-CRE, 2-MCP, 4-MCP, 2,4-DCP, 2,6-DCP, 2,4,5-TCP, 2,4,6-TCP, 2,3,4,5-TeCP, 2,3,4,6-TeCP, PCP and internal standard 4,6-DBC, were applied to the analysis of these compounds in urine. Urine samples, collected from individuals without known exposure to chlorophenols, were examined. Acylated extracts of hydrolyzed urine samples contain complex mixtures of natural organic compounds which are not removed by the simple extraction-cleanup procedure described in Materials and Methods. As shown in Figure 42, it was impossible, using FID, to confirm the presence of the trace chlorophenols present because of the organic background; only the normal urinary phenolic constituents PHE and p-CRE were easily identified. Since m- and p-cresyl acetates are not resolved gas chromatographically using an SP-1240 DA column, it was impossible to ascertain whether a mixture of both of these isomers was present. As shown in Figure 43, an SP-1000 column, which allows the resolution of cresol isomers, can be used to establish that the normal urine samples examined did not contain o- or m-cresol.

A number of methods for GLC analysis of biological fluids have included extensive purification steps on alumina, Florisil and XAD columns (77, 89, 107). Edgerton and Moseman (72) found column cleanup to be essential for the determination of PCP at levels below 30 ppb. Following the analysis of over 400 urine



Peak identification, acetate derivatives of:

1. Phenol
2. o-Cresol
3. p-Cresol
4. 2-Chlorophenol
5. 4-Chlorophenol
6. 2,6-Dichlorophenol
7. 2,4-Dichlorophenol
8. 2,4,6-Trichlorophenol
9. 2,4,5-Trichlorophenol
10. 2,3,4,6-Tetrachlorophenol
11. 4,6-Dibromo-o-cresol
12. 2,3,4,5-Tetrachlorophenol
13. Pentachlorophenol

Figure 42. Trace concentrations of the chlorophenols included in the standard mixture (I) were difficult to distinguish from normal constituents found in acetylated extracts of human urine (II) using FID-GLC. Chromatographic conditions: 1% SP-1240 DA, 75-170°C at 8°/min.

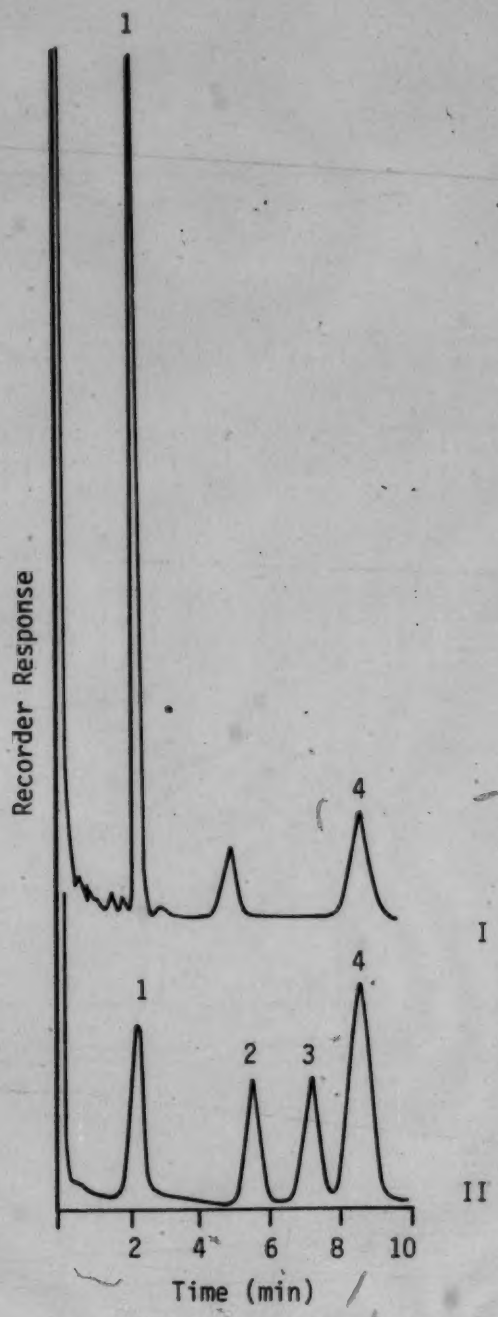


Figure 43. Acetylated extracts of a normal urine sample (I) contained phenol (1) and p-cresol (4); o-cresol (2) and m-cresol (3) were not detected. A standard mixture (II) containing acetylated derivatives of phenol (1), o-cresol (2), m-cresol (3) and p-cresol (4) is shown below. Chromatographic conditions: 0.1% SP-1000, isothermal at 210°C.

samples collected from the general U.S.A. population (305), mean PCP and 2,4,5-TCP concentrations were found to be less than 6 ppb. These concentration levels were at the limits of the specified detection range for the ECD-GLC method used. At these levels, therefore, the phenolics of interest are difficult to distinguish from background or baseline drift using FID or ECD even following lengthy isolation procedures. GLC retention times cannot be used for the unequivocal identification of chlorophenols because other components in the sample may possess coincident retention times. The major limitations of gas chromatographic methods using FID or ECD are inadequate selectivity and specificity. SIM-MS provides more selective detection with sensitivity comparable to ECD and can be used to confirm the presence of trace phenol levels in biological extracts.

The phenols listed in Table XV, added to urine samples at concentrations as low as 1 pmole/mL, could be detected by SIM-MS following aqueous acylation. PCP acetate, for example, was detected by adjusting the MS to monitor only the ion currents of the m/z 266 base peak (due to the loss of ketene from the molecular ion) and its isotope cluster ions m/z 264, 268 and 270. As shown in Figure 44, the MS in total ion current mode (B) produced a response for all the volatile organic components in the acetylated urine extract which entered the ion source. Acetylated PCP, present in the sample at a retention time of 9.6 min, was masked by other constituents. In contrast, using SIM

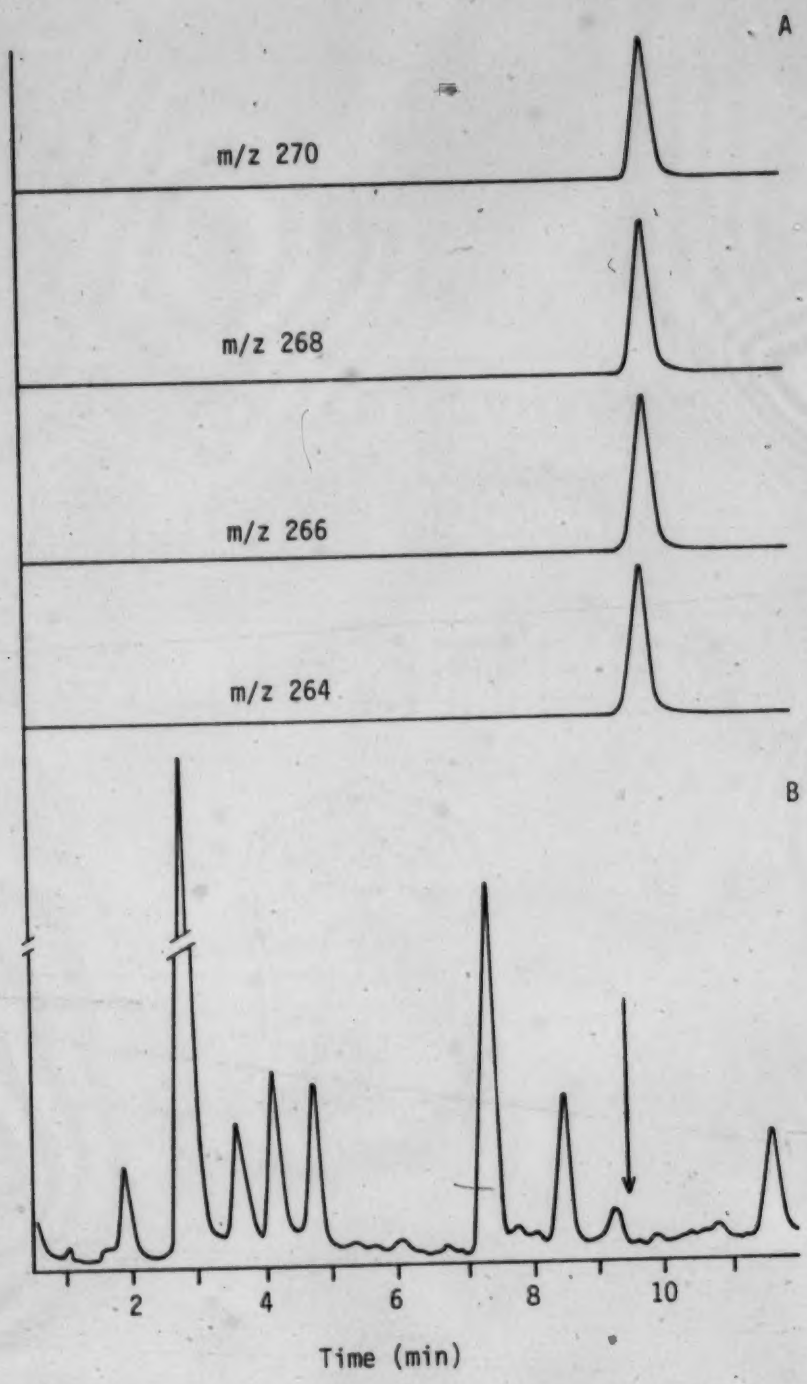


Figure 44. Using SIM-MS (A), the derivatized PCP residue present in the acetylated urine sample could easily be distinguished from the other natural urinary constituents detected by the mass spectrometer in total ion mode (B). Chromatographic conditions: 1% SP-1240, 75-170°C at 8°/min.

(A) a detector response was obtained only for the PCP derivative present in the injected sample. Other compounds in the GLC effluent with similar retention times but which lacked the ions being monitored were no longer detected. PCP was unequivocally identified, since all four ion current peaks were present at the GLC retention time which matched that of the authentic standard.

The SIM profile of a urine sample derivatized with acetic anhydride is shown in Figure 45. The sample, containing added 4,6-DBC internal standard, was found to contain PHE, p-CRE, 2,3,4,6-TeCP and PCP. In order to provide conclusive identification of the presence of a phenolic compound, peaks must be observed at all the monitored diagnostic ions at the expected retention time of the standard compound. Although a number of peaks are present in Figure 45, in the ion current groups II and III, DCPs and TCPs were not present in the sample. In order to confirm the presence of these chlorophenols, peaks must be observed simultaneously at all of the ion currents set to detect them.

At very low chlorophenol concentrations, column adsorption phenomena affected the detection of PCP. After many injections onto the SP-1240 DA column, PCP could no longer be detected in extracts of samples spiked with the standard mixture of thirteen phenols. Wu et al. (178) reported similar effects for analysis of PCP in extracts of rainbow trout tissue. It was observed in the present procedure that PCP could no longer be detected if the column became contaminated with extraneous sample

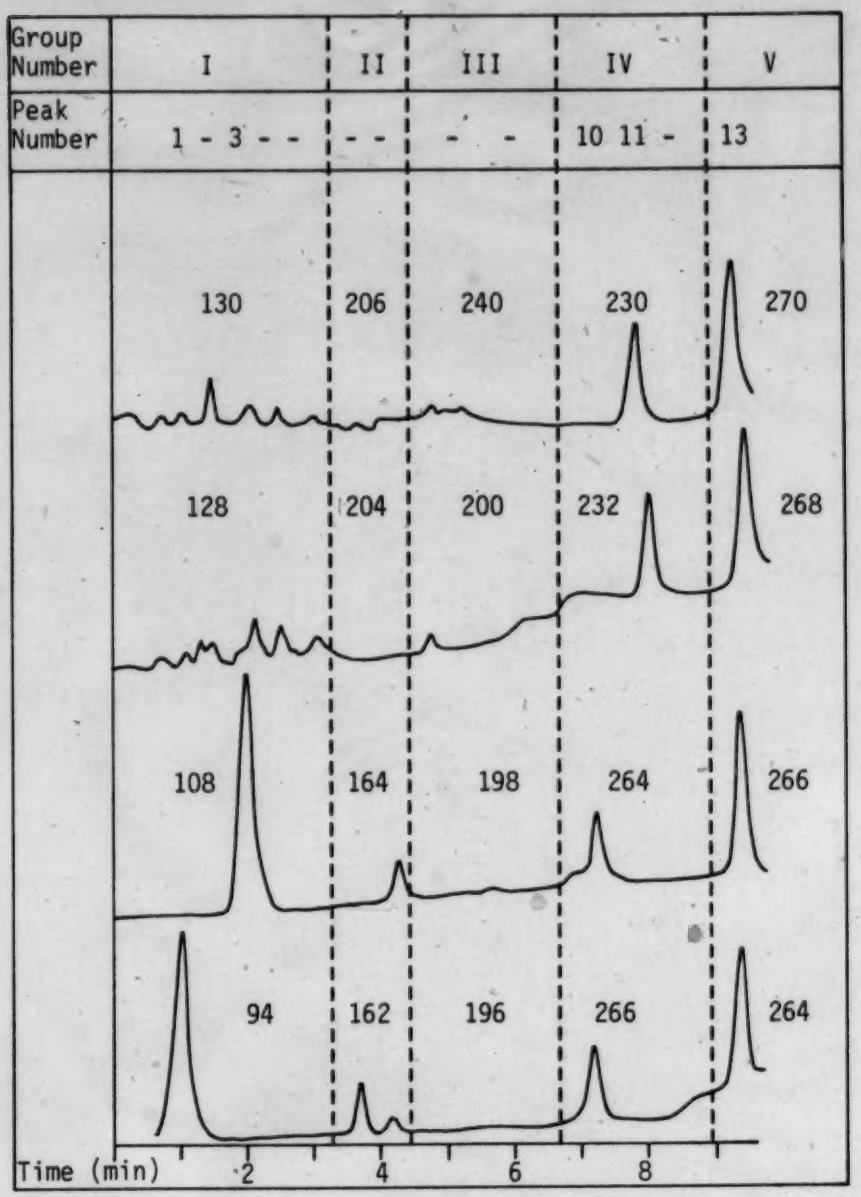


Figure 45. SIM-MS analysis of an acetylated urine sample identified the presence of phenol (1), p-cresol (3), 2,3,4,6-tetrachlorophenol (10) and pentachlorophenol (13). 4,6-Dibromo-o-cresol (11) was added as internal standard. Ion groups and peak numbers correspond to the SIM-MS program outlined in Table XVI. Chromatographic conditions: 1% SP-1240 DA, 75-170°C at 8°/min.

constituents. Adsorption effects of the other twelve phenols included in this study were not as pronounced as those experienced with PCP. If the glass wool plug and the first few inches of column packing material were replaced regularly, losses of PCP on column were minimized. Of the twenty-five urine samples analyzed by SIM-MS following derivatization with both propionic and acetic anhydride, more than 90% contained detectable levels of PCP.

2. Environmental Water Samples

a) Study of Nitrophenols in the Athabasca River

Methyl and ethyl parathions (Table I), among the most commonly used biodegradable pesticides in North America (188), are degraded to 4-NP. Both 2-NP and 4-NP are listed by the World Health Organization (52) and the EPA (293-295) as priority pollutants. The parathion pesticides, as well as other industrial chemicals and dyes (20, 22, 192), may contribute to the concentration of the nitrophenols found in river waters. Athabasca River samples were examined for the presence of 2- and 4-NP. When 750 mL samples, containing 0.1 μmoles of 1-NAP internal standard, were treated with acetic anhydride, peaks corresponding to the retention times of acetylated 2- and 4-NP were not detected. Phenolic compounds, other than traces of PHE, o-CRE and p-CRE, were not detected using SIM-MS. If the Athabasca River contained nitrophenols, they were present at

concentrations well below the EPA upper limits of 250 and 100 µg/L for 2- and 4-NP, respectively, in drinking water (Table XI). The U.S.S.R. has introduced much more stringent guidelines for nitrophenol content in drinking water. Based on sanitary and toxicological grounds, concentration limits of 100, 60 and 20 µg/L (1, 52) have been set for 1-NAP, 2-NP and 4-NP, respectively. Had samples of Athabasca River water contained these compounds in excess of the U.S.S.R. limits this could easily have been confirmed using the aqueous acetylation procedure described here.

The persistence of organic compounds in water is very dependent on the aquatic system and its indigenous microflora. In marine water samples (306) carbaryl completely disappeared after a seventeen day incubation at 20°C with 43% conversion to 1-NAP. 1-NAP is as toxic to a number of aquatic species (307) as the original pesticide, carbaryl. The stability of phenolic pesticide metabolites is important, therefore, not only because these compounds are markers for monitoring pesticide contamination but also because of their inherent toxicity. A recent review of the literature concerning phenolic compounds in water (1) contained few references dealing with the fate of phenolics in aqueous ecosystems. In the present study (Figure 46), when 1 µmole/L of 2-NP, 4-NP and m-CRE were each added to Athabasca River water, the concentrations of both nitrophenols remained virtually unchanged over the 2-week sampling period, whereas the concentration of m-CRE rapidly declined within three

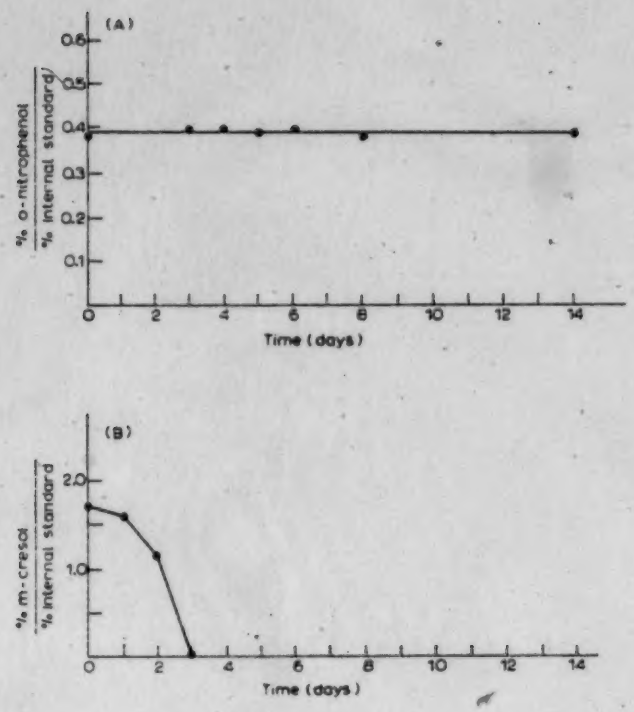


Figure 46. The metabolism of (A) 2-nitrophenol and (B) m-cresol added to Athabasca River water each at concentrations of 1 µmole/L. The amount of original phenol remaining was calculated using internal standard addition.

days. These results compare favourably to those reported by Chambers et al. (48) for the degradation of phenols and other aromatic compounds by organisms isolated from muds at a catalytic cracking plant waste lagoon. It was reported that o-, m- and p-CRE were oxidized more readily than dimethyl-, chloromethyl-, nitromethyl-, chloronitro-, nitro-, and aminophenols.

b) Identification and Quantitation of Phenols in North Saskatchewan River Water and Edmonton Municipal Snow Dumps

The major objective of this study was to assess the effect of melting snow, from municipal snow dumps located along the river, on the concentration of phenols in North Saskatchewan River water. FID-GLC and GLC-MS were used to analyze water and melted snow samples from municipal snow dumps for the presence of the forty-one phenolics listed in Table XI. The Department of Health and Welfare (Canada) (36) has emphasized that chlorophenols are of particular concern in the assessment of drinking water quality. Therefore, in addition to FID-GLC and GLC-MS, river water and snow dump samples were more thoroughly screened for a series of nine chlorophenols using SIM-MS.

Phenolic compounds in water samples undergo both chemical and biological decomposition with storage (307-309). Carter and Huston (308) reported that 40% of the PHE added to treated sewage samples was lost during 24 hr storage at 4°C. Addition of acid, alkali or copper sulfate plus phosphoric acid allowed the storage of samples for up to eight days at 4°C without

decomposition. In this study, all of the samples were collected in duplicate; one set was preserved with $CuSO_4 \cdot 5H_2O$ and H_3PO_4, while no preservatives were added to the second set of samples. Both preserved and unpreserved samples, analyzed as soon as possible after collection, gave identical chromatograms. The presence of the $CuSO_4$ preservative did not interfere with the effectiveness of the aqueous acetylation procedure.

When North Saskatchewan River samples from up- and downstream of a snow dump site were acetylated following the addition of internal standard 4,6-DBC, gas chromatograms similar to those shown in Figure 47 were obtained for all samples. The only phenolic compound which could be detected by FID-GLC in samples collected both up- and downstream of the snow dump site on March 4, 5 and 6, 1981, as well as in the composite samples, was PHE itself. The concentration of PHE found in the North Saskatchewan River ranged from 0.25-0.5 µg/L; concentrations in samples from up- and downstream of the snow dump site were similar. As shown in Table XIX, SIM-MS analysis of the river water samples further identified traces of o-CRE, p-CRE and PCP.

A duplicate set of river samples, comparable to those collected in this study for GLC analysis, were sent to a private consulting firm for "phenol" analysis. The colorimetric 4-AAP test (256) was performed. River water samples from up- and downstream of the snow dump site were reported to contain "phenolics" at concentrations of 3 µg/L and < 1 µg/L, respectively. These "total phenolics" results, as expected,

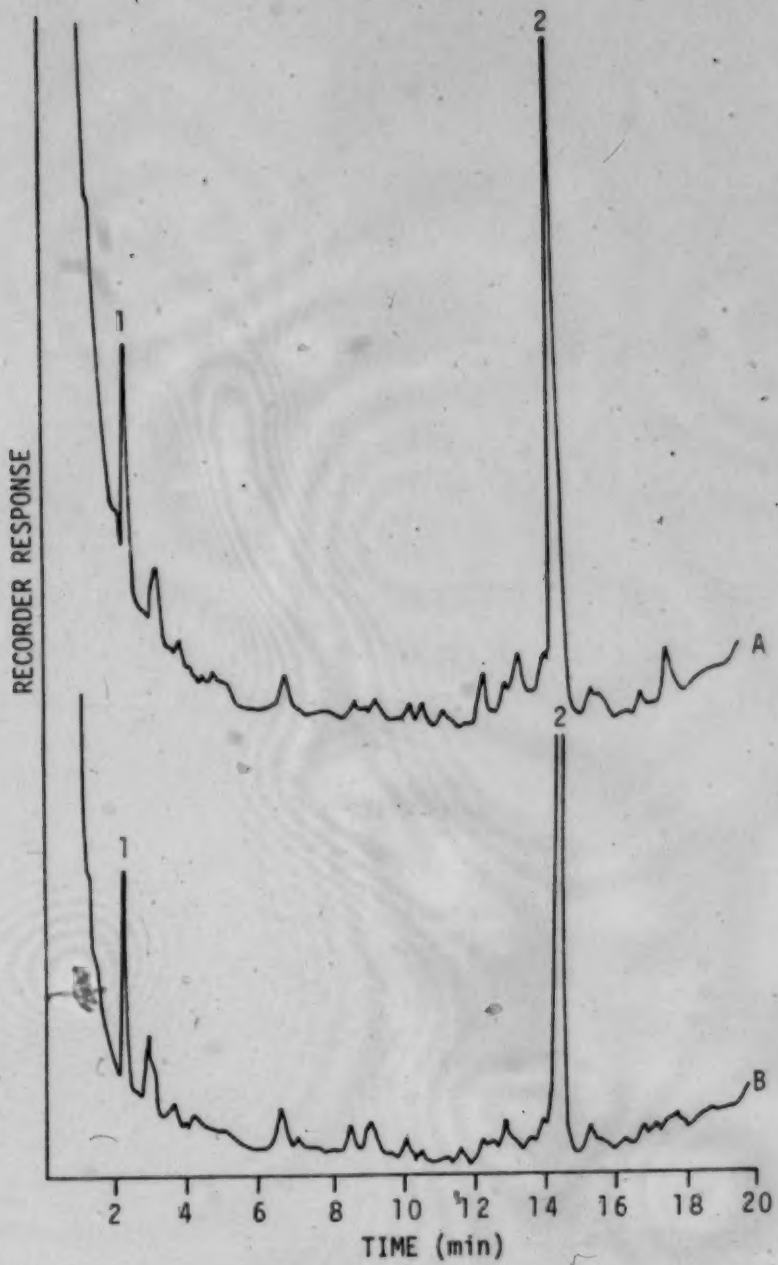


Figure 47. Gas-liquid chromatograms of acetylated extracts of North Saskatchewan River Water: (A) Composite sample from downstream (east) of the snow dump site. (B) Composite sample from upstream of the snow dump site. Only phenol (1) was identified by FID-GLC. 4,6-Dibromo-o-cresol (2) was added to the sample as the internal standard. Chromatographic conditions: 1% SP-1240 DA, 75-170°C at 4°/min.

Table XIX. Chlorinated Phenols Identified in the North Saskatchewan River and Snow Dump Sites Using Selected Ion Monitoring-Mass Spectrometry

Phenol	N. Saskatchewan River		Snow Dump		
	Upstream of Snow Dump	Downstream of Snow Dump	SDX-1	SDX-2	SDX-3
phenol	+	+	+	+	+
o-cresol	+	+	+	+	+
p-cresol	+	+	+	+	+
2-chlorophenol	-	-	-	-	-
4-chlorophenol	-	-	-	-	-
2,4-dichlorophenol	-	-	+	+	+
2,6-dichlorophenol	-	-	-	-	-
2,4,6-trichlorophenol	-	-	-	-	-
2,4,5-trichlorophenol	-	-	-	-	-
2,3,4,5-tetrachlorophenol	-	-	-	-	-
2,3,4,6-tetrachlorophenol	-	-	+	+	+
pentachlorophenol	+	+	+	+	+

+ = present; - = absent

were considerably higher than those obtained by the specific quantitative assay of PHE using FID-GLC. The many complex, large molecular weight phenolic substances not detected gas chromatographically and nonphenolic interfering substances which react with 4-AAP may account for the discrepancy in reported phenol values.

Results of both GLC and colorimetric methods confirmed that the concentration of phenolics in the North Saskatchewan River was not detectably altered by runoff from the snow dump. If an increase in phenol concentration had been observed downstream of the snow dump, colorimetric analysis could not have conclusively incriminated the snow dump as the source of these phenols. In contrast, the GLC detection of specific phenolic compounds in snow dump and down-river water samples, which were absent from up-river samples, could have pinpointed the source of pollution.

Figure 48 shows the FID gas chromatogram obtained from an acetylated extract of a snow sample. PHE, o-CRE, p-CRE and two peaks identified as either ethyl- or dimethylphenols were detected in all of the snow dump samples. The two alkylphenols could not be unequivocally identified since several isomers of ethyl- and dimethylphenols have similar GLC retention times as well as similar mass spectra. Table XX summarizes the concentrations of PHE, o-CRE and p-CRE found in the three snow dump sites analyzed. In all three samples, p-CRE was the major simple phenolic constituent. Using SIM-MS (Table XIX), trace levels of 2,4-DCP, 2,3,4,6-TeCP and PCP were also identified.

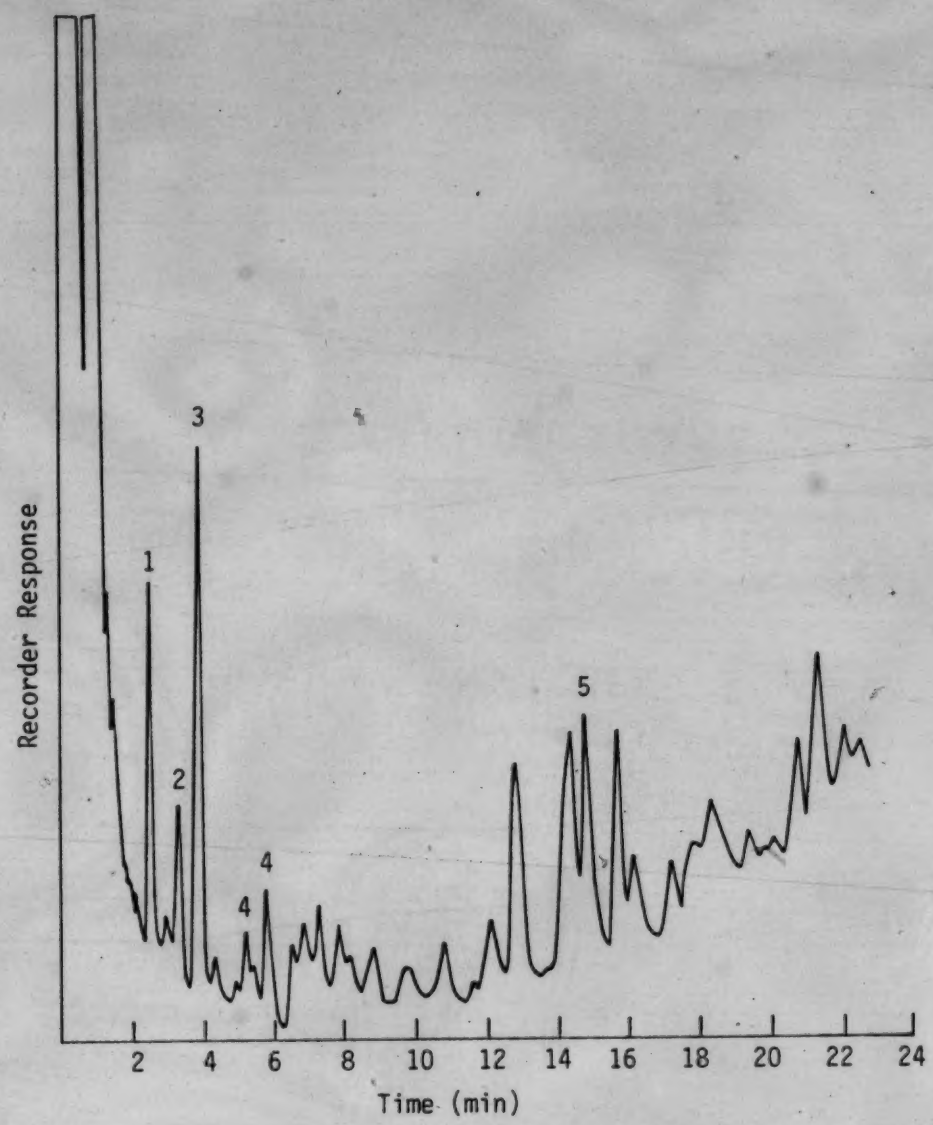


Figure 48. Gas-liquid chromatogram of an acetylated extract of a snow dump site (sample SDX-81-1). The sample contained phenol (1), o-cresol (2), p-cresol (3) and two unidentified dimethyl- or ethylphenols (4). Internal standard 4,6-dibromo-o-cresol (5) was added to the sample prior to acetylation.

Table XX. Concentrations of Phenols (in µg/L = ppb) Found in Snow Dump Samples

Phenolic Compound	Site SDX-1	SDX-2	SDX-3
phenol	3.3	4.7	4.7
o-cresol	1.6	3.7	2.2
p-cresol	6.5	9.7	10.8

The simple phenols detected in snow samples may have been derived from a number of different sources. Roumeliotis et al. (29) identified PHE, CREs, DMPs and trimethylphenols in automobile engine exhaust. These phenols are also associated with industrial effluents from petrochemical plants or petroleum refineries (22-27). The chlorophenols are commonly introduced as decomposition products of agricultural pesticides (Table II). Alternatively, leaf litter and soil are natural sources of simple phenolic compounds.

The river water downstream of the snow dump did not reflect the phenolic composition of the melted snow samples. The flow volumes of the North Saskatchewan River were apparently sufficient to dilute the concentration of phenolics in the incoming discharge from the melting snow.

c) Analysis of Simple Phenols in Raw and Treated North Saskatchewan River Water

During the past few years, a great deal of public concern has been focused on the quality of drinking water and, in particular, on the presence of organic contaminants in water used for human consumption. Many compounds, including aliphatic acids, aromatic hydrocarbons, amines, lower fatty acids, esters, aldehydes, ketones, sulphides, mercaptans and phenols, have been linked to taste and odour problems in potable water (1). Several phenolic compounds, particularly the chloro- and dichlorophenols, create detectable odours and "medicinal" tastes at ppb levels and below (36).

Each year, during the spring runoff period, drinking water quality problems are experienced by the City of Edmonton. The objectionable odour and taste of treated drinking water has often been attributed to "phenolics", however, the exact nature of the compounds responsible has not been conclusively determined. In this study, raw North Saskatchewan River water samples, collected in the winter, spring and early summer, were analyzed for the presence of the forty-one phenols shown in Table XI. It has been reported (35, 310) that chlorophenols may be formed during chlorination processes. Treated water samples were, therefore, also analyzed, in conjunction with raw water samples, in order to determine whether compounds not present in raw source water were formed during the treatment process.

When water samples (3 L) were acetylated and extracted, gas chromatograms similar to those shown in Figure 47 were obtained for both raw and treated water samples on all three sampling dates. Only phenol itself was present in high enough concentrations to be detected by FID-GLC. Using SIM-MS, trace levels of o-CRE, p-CRE, 2,3,4,6-TeCP and PCP were also unequivocally identified. Concentrations for all of the phenolic components were estimated to be below 0.1 μg/L. Further, no simple phenolic compounds were detected in treated waters which were not present in the corresponding raw river water samples. A more extensive sampling program would be required to confirm that phenolic compounds cannot be implicated in the drinking water taste and odour problems during spring runoff.

d) Analysis of Phenols in Industrial Waters

Syncrude Canada Ltd. currently operates an oil sands extraction plant based on an open pit mining approach in the Athabasca oil sands; the surface mining site is shown in Plate 1. The production of synthetic crude oil from raw bitumen (Plate 2) is accomplished in three basic steps, oil sand mining, bitumen extraction and bitumen upgrading. In the extraction process, mined oil sand is agitated with the addition of water and steam. Two sources of water, recycled process water and fresh water, are used. Sodium hydroxide is added to raise the pH of the slurry to approximately 8.5 and the temperature is increased to 80°C. The bitumen is extracted and a slurry of hot water and "tailings" (50% solids by weight) is transported by pipeline to the tailings pond for storage (Plate 3). The tailings pond was created in compliance with a "zero discharge" policy in which no process-affected waters may be directly released into the surrounding aquatic environment. As shown in Plate 4, accumulated process waters now occupy an area of approximately 15 km^2 and will eventually expand to cover 28 km^2.

The toxic process waters are essentially contained within a large and continuously growing artificial system. Any leakage from the dykes surrounding the tailings pond is controlled by a drainage system (Plate 5) which eventually pumps the escaping water back into the pond. Evaporation, precipitation and percolation into surrounding aquifers are three processes which cannot be strictly controlled. It is important to ensure that

Plate 1. Syncrude Canada Ltd. oil sands mining site, north of Fort McMurray, Alberta.

Plate 2. Raw bitumen (right) is converted to the final product, synthetic crude (left), by a series of upgrading steps.

Plate 3. Process effluent discharge point into the tailings pond.

Plate 4. Syncrude Canada Ltd. tailings pond (Ref. 312).

Physical Characteristics

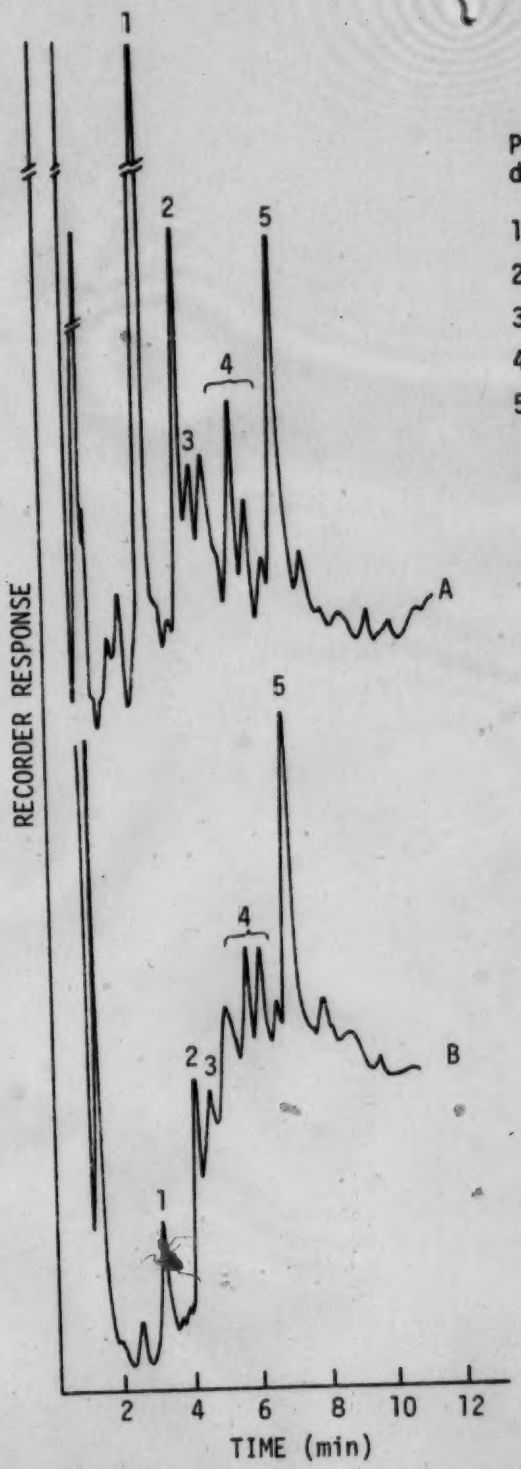
	Present (1981)	Projected
Area	15 km^2	28 km^2
Water Depth		
Deepest	25 m	60 m
Average	10 m	35-40 m
Volume	150 x 10^6 m^3	10^9 m^3
Rate of Growth (per month)	3 x 10^6 m^3	3 x 10^6 m^3

Plate 5. Tailings pond drainage water outlets feed leakage into a collector ditch.

tailings effluents leak neither into surface water bodies nor into the ground water regime. In this study, simple phenols, present in oil sand process-affected waters, were used as tracer compounds to follow the possible seepage of the tailings pond into surrounding natural water systems.

FID-gas chromatograms of tailings pond water samples, acetylated and extracted 6 hr and 48 hr following sample collection are shown in Figure 49 A and 49 B, respectively. PHE, o-, m- and p-CRE and four different dimethyl- or ethylphenols were identified in the fresh tailings pond sample (Figure 49 A). The 48 hr storage period at 4 °C dramatically altered the phenolic composition of the tailings pond water; less than 10% of the PHE concentration originally in the sample remained. The loss of phenolic compounds from other unpreserved tailings pond samples was variable, but substantial in all cases. The rapid metabolism of simple phenols present in the tailings pond waters clearly demonstrated the importance of expedience in analysis of such samples. The gas chromatograms shown in Figure 49 were obtained using an OV-101 column. It was, therefore, not possible to resolve m- and p-CRE; the ethyl- and dimethylphenol isomers present could also not be unequivocally identified. Using SP-1000 (Figure 50) it was demonstrated that all three cresol isomers were present in the tailings pond waters.

FID-gas chromatograms obtained following acetylation and extraction of process-affected water samples collected from the



Peak identification, acetate derivatives of:

1. phenol
2. o-cresol
3. m-/p-cresol
4. dimethyl- or ethylphenols
5. 4-bromophenol (internal standard)

Figure 49. Gas-liquid chromatogram obtained following aqueous acetylation and extraction of 100 mL of tailings pond water 6 hr (A) and 48 hr (B) following sample collection. Chromatographic conditions: 5% OV-101, 85-220°C at 8°/min.

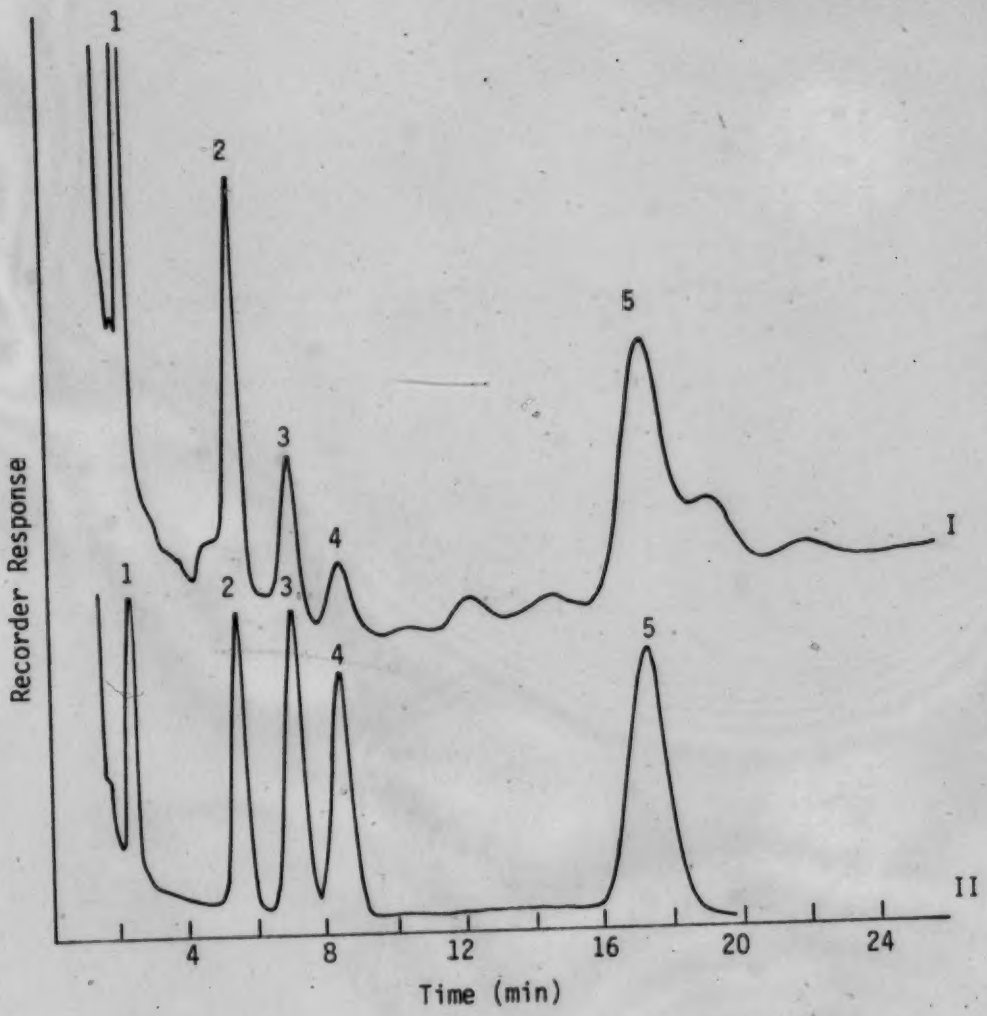


Figure 50. Acetylated extracts of a 100 mL tailings pond water sample (I) contained acetate ester derivatives of phenol (1), o-cresol (2), m-cresol (3) as well as p-cresol (4). Internal standard 4-bromophenol (5) was added to the sample before analysis. A standard mixture of acetylated phenol, cresols and 4-bromophenol is shown below (II). Chromatographic conditions: 0.1% SP-1000, 210°C isothermal.

dyke drainage, collector ditch and catchment basin are shown in Figure 51, Figure 52 and Figure 53, respectively. These samples contained only traces of PHE and o-, m- and p-CRE; the presence of these compounds was confirmed using SIM-MS. None of the other simple alkylphenols, identified in tailings pond water, were present at detectable levels. Chromatograms similar to those in Figure 51, Figure 52 and Figure 53 were obtained following analysis of all natural surface and groundwaters included in this study.

The SIM-MS program shown in Table XVI was applied to the analysis of chlorophenols in acetylated sample extracts in this study. While trace levels of PCP and 2,3,4,6-TeCP have been identified in North Saskatchewan River water, City of Edmonton drinking water and urine samples from the general population of Edmonton, no chlorophenol residues could be detected in the samples listed in Table XXI.

The quantitative results summarized in Table XXII clearly indicate that simple phenols are very effectively contained within the tailings pond. Concentrations of PHE, o-CRE and m-/p-CRE in drainage water samples were comparable to those observed in natural surface water samples. In the tailings pond, concentrations of PHE, o-CRE and m-/p-CRE decreased sharply with increasing pond depth. It has been reported (311) that the concentration of suspended solids within the pond increases with depth, as the particulate matter in the extraction process effluent slowly settles to the pond bottom.

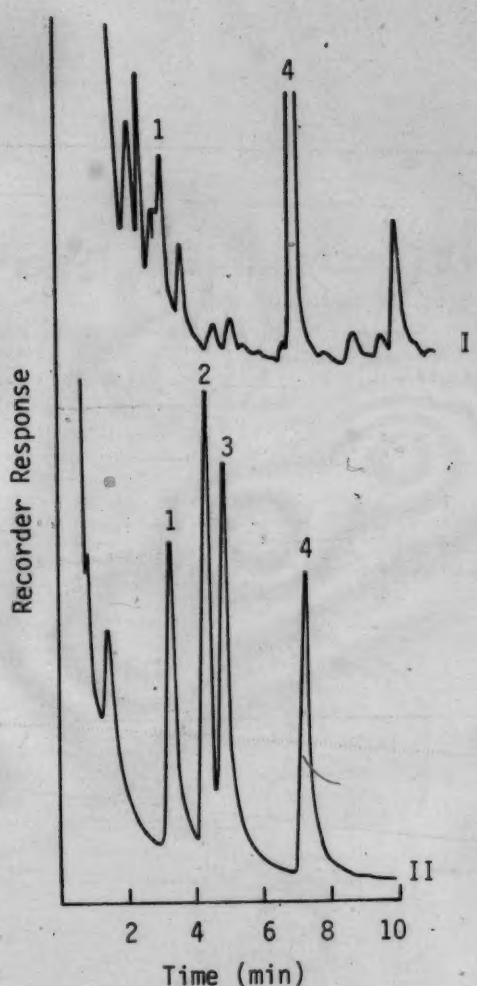


Figure 51. Acetylated extracts of a 1 L dyke drainage water sample (I) contained only traces of phenol, o-cresol and m-/p-cresol; 4-bromophenol was added as internal standard. An acetylated standard mixture (II) containing derivatives of phenol (1), o-cresol (2), p-cresol (3) and 4-bromophenol (4) is shown below. Chromatographic conditions: 5% OV-101, 75-220°C at 8°/min.

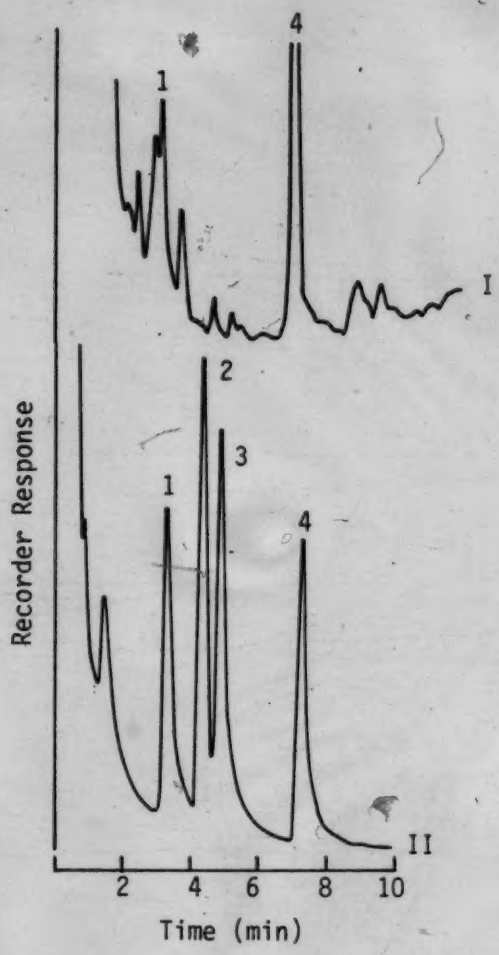


Figure 52. Acetylated extracts of a 1 L collector ditch water sample (I) contained only traces of phenol, o-cresol and m-/p-cresol; 4-bromophenol was added as internal standard. An acetylated standard mixture (II) containing derivatives of phenol (1), o-cresol (2), p-cresol (3) and 4-bromophenol (4) is shown below. Chromatographic conditions: 5% OV-101, 75-220°C at 8°/min.

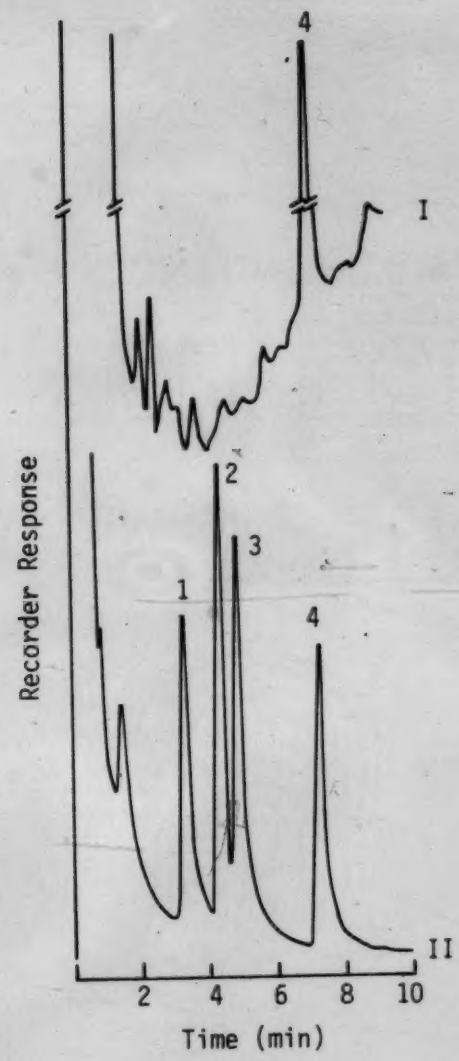


Figure 53. Acetylated extracts of a 1 L catchment basin water sample (I) contained only traces of phenol, o-cresol and m-/p-cresol; 4-bromophenol was added as internal standard. An acetylated standard mixture (II) containing derivatives of phenol (1), o-cresol (2), p-cresol (3) and 4-bromophenol (4) is shown below. Chromatographic conditions: 5% OV-101, 75-220°C at 8°/min.

Table XXI. Phenols Identified in Process-Affected and Natural Waters Using Selected Ion Monitoring-Mass Spectrometry (+ = present; - = absent)

Phenol	Sample Type					
	Tailings Pond[1]	Dyke Drainage[2]	Collector Ditch[3]	Catchment Basin[4]	Piezometers[5]	Natural Surface Water[6]
Phenol	+	+	+	+	+	+
o-Cresol	+	+	+	+	+	+
m-/p-Cresol	+	+	+	+	+	+
2-Chlorophenol	-	-	-	-	-	-
4-Chlorophenol	-	-	-	-	-	-
2,6-Dichlorophenol	-	-	-	-	-	-
2,4-Dichlorophenol	-	-	-	-	-	-
2,5-Dichlorophenol	-	-	-	-	-	-
2,4,6-Trichlorophenol	-	-	-	-	-	-
2,4,5-Trichlorophenol	-	-	-	-	-	-
2,3,4,6-Tetrachlorophenol	-	-	-	-	-	-
2,3,4,5-Tetrachlorophenol	-	-	-	-	-	-
Pentachlorophenol	-	-	-	-	-	-

1. Sample #8-14.
2. Sample #1-4.
3. Sample #5.
4. Sample #6, 17.
5. Sample #18, 20, 21, 23-27.
6. Sample #7, 16, 19, 22, 29.

Table XXII. Quantitative Analysis of Phenol and Cresols in Process-Affected and Natural Water Samples

Source	Sample[1] Number	Sample[1] Description	Concentration[2] (μmoles/L) Phenol	o-Cresol	m-/p-Cresol
PROCESS-AFFECTED WATER:					
Tailings Pond (Depth Profile)	8	2 m	1.67	1.06	0.50
	9	6 m	1.08	0.90	0.38
	10	10 m	0.95	0.51	0.27
	11	15 m	0.33	0.38	0.23
	12	2 m	1.55	1.20	0.57
	13	6 m	1.62	0.58	0.24
	14	10 m	0.75	0.60	0.23
Tailings Pond Drainage	1,2,3,4	Dyke drainage	0.02	<0.01	<0.01
	5	Collector ditch	<0.01	<0.01	<0.01
	6,17	Catchment basin	<0.01	<0.01	<0.01
NATURAL WATER:					
Groundwater (Piezometers)	18,24	OW12R	<0.01	<0.01	<0.01
	20,25	OW3R	<0.01	<0.01	<0.01
	21	T-2	<0.01	<0.01	<0.01
	23	OW27R	<0.01	<0.01	<0.01
	26	T-14	<0.01	<0.01	<0.01
	27	OW18R	<0.01	<0.01	<0.01
Surface Water	7,16	Sand Pit	<0.01	<0.01	<0.01
	22	Mildrid Lake	<0.01	<0.01	<0.01
	19	Beaver Creek	<0.01	<0.01	<0.01
	29	Natural drainage over oil sand	0.04	<0.01	<0.01
Hot Water Extracts	28	Bench scale extraction	<0.01	<0.01	<0.01

1. Samples 8-11 and 12-14 were taken at two different locations within the tailings pond.
2. Exact concentrations less than 0.01 μmoles/L are not specified.

The decrease in PHE, o-CRE and m-/p-CRE concentrations in tailings pond water with depth is probably linked to the adsorption of these compounds to the settling particulate matter. Using a 4-AAP colorimetric test, total phenolic concentrations in the tailings pond were reported (311) to be 0.15-0.4 mg/L. These concentration levels are similar to those shown in Table XXII. At a 2 m depth, for example, the tailings pond was found to contain a combined total concentration of 0.33 mg/L PHE, o-CRE plus m-/p-CRE. The tailings pond, however, also contains four dimethyl- and ethylphenols whose concentrations were not estimated in this study. It is interesting that the distinct phenol concentration gradient evident in Table XXII could not be demonstrated using the colorimetric 4-AAP total phenols method (311). Sample 28 in Table XXII was prepared by the extraction of oil sand on a bench scale simply using hot water. Sample 29 was collected from a natural stream flowing over exposed oil sand. Though sample 29 contained slightly higher concentrations of PHE than other natural water samples, the high concentrations of alkylphenols, cresols and PHE contained in tailings pond water were absent from both samples 28 and 29. This might suggest that these phenolic compounds are produced during the hot caustic extraction of oil sand.

Groundwater samples, collected from piezometers, were similar in phenolic content to natural surface waters; no evidence of tailings pond leakage was detected. The rate of intrusion of tailings pond water was expected to be minimal

because of the relatively impermeable layer of sludge and fines which has accumulated on the bottom of the pond (311).

Despite the absence of tailings pond phenols from groundwater samples, the existence of leakage cannot be entirely excluded. It is possible that the percolation of tailings pond water has been masked by groundwater dilution effects or the rapid metabolism of the simple phenols analyzed in this study. Though the metabolism of phenolics is rapid in stored tailings pond water, Defino and Dube (256) reported the persistence of PHE in groundwater for nineteen months following an accidental spill. In addition, attempts to dilute the PHE concentration with 360,000 L of water was completely ineffectual. In the reported incident, PHE was used to monitor and assess the impact of the spill. Although leakage from the tailings pond was not demonstrated in this study, the aqueous acetylation method was successfully applied to the analysis of trace phenol concentrations in industrial and natural water systems.

3. Forensic Samples

In this study, the simple one-step aqueous acylation method was applied to the illicit drug screening of street dosage preparations for the presence of morphine. Comparison of the gas chromatograms obtained following extraction of samples without prior derivatization (Figure 54 B) to an authentic standard (Figure 54 A) confirmed that heroin was not present. The samples were acylated in aqueous

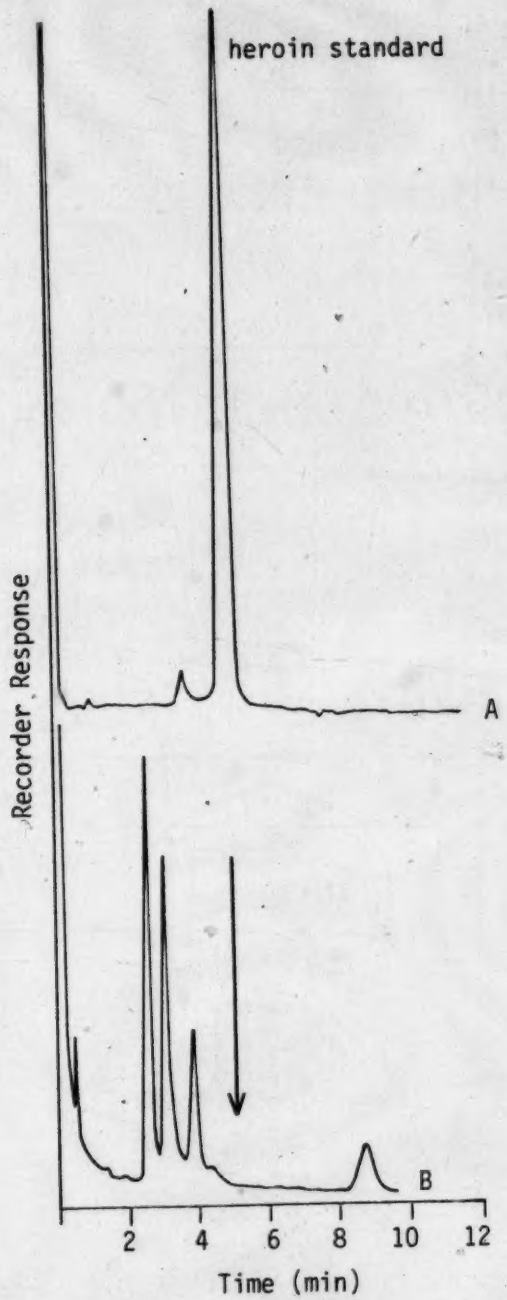


Figure 54. Comparison of the gas chromatogram obtained following extraction of an illicit preparation (B), prior to derivatization, with an authentic standard containing heroin (A) confirmed that heroin was absent from the sample. Chromatographic conditions: 3.8% OV-17 (1.68 m), 290°C isothermal.

solution with propionic anhydride and the presence of ^{3}O-propionylmorphine indicated that the illicit preparations contained morphine (Figure 55). The presence of morphine was further confirmed by the derivatization of the samples with acetic anhydride, which produced a peak corresponding to ^{3}O-acetylmorphine. Both ^{3}O-acylmorphine derivatives, identified in the illicit preparations, were unequivocally characterized by comparison of their mass spectra to those obtained for authentic morphine standards similarly acylated (Figure 39).

Quantitative analysis of propionyl morphine derivatives, using benzo(a)pyrene as internal standard, determined that the eight illicit samples examined contained 5.8-16.2% (W/W) morphine. The aqueous acylation method using propionic anhydride was successfully applied to the quantitative determination of morphine in street drug preparations. Although acetic anhydride could not be used for the analysis of samples containing any combination of morphine, ^{3}O-acetylmorphine, ^{6}O-acetylmorphine and heroin, in this study aqueous acetylation provided additional evidence which further corroborated the presence of morphine in the illicit drug samples examined.

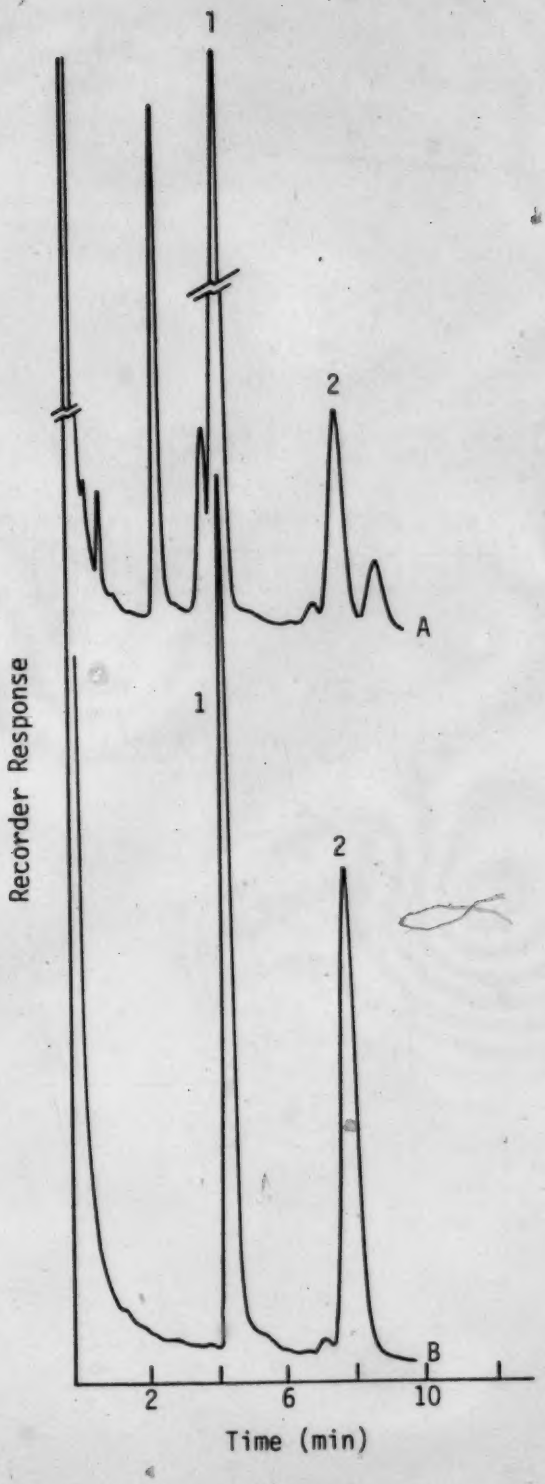


Figure 55. Gas-liquid chromatograms obtained following derivatization of an illicit preparation (A) and authentic morphine standard (B) with propionic anhydride in aqueous solution. Peaks 1: 3-O-propionylmorphine; 2: benzo(a)pyrene. Chromatographic conditions: 3.8% OV-17, 290°C, isothermal.

CHAPTER VI

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